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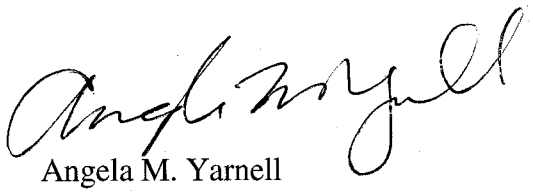

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Abstract

Title of Thesis: A Neurobehavioral Phenotype of Blast Traumatic Brain Injury and Psychological Stress in Male and Female Rats

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Current wars are taking physical and psychological tolls on American service members. Many Warriors return with traumatic brain injuries (TBI) and mental health problems. Psychological stress that Warriors experience might contribute to the TBI-mental health problem relationship. This experiment examined blast injury and psychological stress in male and female rats. A blast over pressure paradigm (BOP) was used to model blast exposure. A combination of predator and environmental stressors was used to stress the rats. A measure of neurobehavioral functioning (NSS-R) and measures of activity were collected. The blast exposure via the BOP model increased NSS-R scores in blasted animals. Psychological stress attenuated negative effects of blast-induced injury on neurobehavioral function in male rats, but potentiated negative neurobehavioral effects of blast-induced injury in female rats. Future research could use the BOP model to investigate mechanisms that underlie these neurobehavioral effects and potential preventive and treatment interventions.

A Neurobehavioral Phenotype of Blast Traumatic Brain Injury and Psychological Stress in Male and Female Rats

by

Angela M. Yarnell

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Sentiment is not a skill that is taught at Officer Basic School or graduate school. I guess that is why it has been difficult to put into words the gratitude that I feel for the people who have contributed to this project and to my education over the last two years. But, I will try.

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It may not be my lack of training in sentiment that makes it difficult for me to phrase how I feel. I think that it may just be that I am so blessed to be surrounded by remarkable friends, supporters, and mentors that there are no words to express my gratitude. So, perhaps I should simply praise the Lord for blessing me with these individuals and giving me the strength I need to succeed every day. “Thank you God for the blessings you have provided me and the strength that you grant me every day. Amen.”

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The wars in Iraq and Afghanistan are taking physical and psychological tolls on American service members. Fifteen to thirty percent of returning Warriors have sustained a traumatic brain injury (TBI; Hoge et al., 2008; Okie, 2005; Survivability/Vulnerability Information Analysis Center, 2009; Tanielian et al., 2008; Terrio et al., 2009; Warden, 2006). One third of returning Troops suffer from mental health problems, such as anxiety, depression, and post-traumatic stress disorder, and these conditions are often comorbid with TBI (Hoge et al., 2008; Lew et al., 2009; Sayer et al., 2009; Schell & Marshall, 2008; Tanielian & Jaycox, 2008; Tanlielian et al., 2008). According to Vice Chief of Staff for the Army, General Peter Chiarelli, 66% of returning Warriors are suffering from TBI and/or PTSD (Chiarelli, 2011). Warriors are exposed to unique psychological stress during deployment that could alter the effects of TBI and moderate its relationship with these mental health problems. It is important to determine whether the relationship between TBI and mental health problems is causal to help develop optimal strategies and interventions to prevent and treat these conditions.

The purpose of the present study was to examine the effects of blast-induced TBI (bTBI) on neurobehavioral measures and activity in rats to quantify a behavioral phenotype that can be used to: (1) model the human response; (2) determine whether other variables (e.g., stress, time) attenuate or exacerbate the bTBI; and (3) examine sex differences. This experiment used behavioral outcome measures that model the human condition (neurobehavioral functioning, anxiety-like and depression-like behaviors) to create this phenotype. Through this experiment it was possible to segregate certain key variables: the injury alone; the stress associated with deployment and injury; the sex of the patient; and the time course of the variables.

There are a number of questions about TBI that this experiment set out to answer. What are the effects of bTBI on behavior? Are the deleterious effects of bTBI acute or longer lasting? Is there a link between bTBI and mental health problems? Does psychological stress moderate this relationship? Are there any sex differences? This paper presents the background information currently available for bTBI, stress and Warrior stress, bTBI and mental health problems, bTBI and sex, bTBI and time, and the value of animal models for experiments in bTBI. Then the specific aims, hypotheses, methods, results, discussion, and conclusion from the experiment are presented.

Blast TBI

Many service members are surviving previously fatal injuries because of improved trauma interventions and better protective equipment (Regan, 2004; Tanielian & Jaycox, 2008; Warden, 2006). It has become evident that some service members who survive their injuries are suffering from lasting effects (Martin et al., 2008). TBI, one of the signature wounds of the Global War on Terrorism, is one type of injury with long-term effects. According to recent reports, 15-28% of returning Veterans have sustained a TBI (Hoge et al., 2008; Okie, 2005; Survivability/Vulnerability Information Analysis Center, 2009; Tanielian et al., 2008; Terrio et al., 2009; Warden, 2006). In the last 11 years, 220, 430 cases of TBI were reported to the DoD with 77% meeting criteria for mild TBI (Armed Forces Health Surveillance Center, 2011). Reports indicate that 88% of these TBIs are closed head injuries and 68% result from exposure to blast (Hoge et al., 2008; Martin, Lu, Helmick, French, & Warden, 2008; Wojcik et al., 2010; U.S. Department of Defense and Department of Veterans Affairs, Traumatic Brain Injury Task Force, 2008). Exposure to blast was highest in 2010 (J-MHAT, 2011), compared to

reports from 2003 to 2009, which may have resulted in the recent rise in TBIs (Armed Forces Health Surveillance Center, 2011).

bTBI is more complex than other forms of TBI (DIVBIC, 2011) and may be different enough to constitute its own category (Cernak & Noble-Haeusslein, 2010; Ling et al., 2009). A blast wave alone may cause damage without leaving any external sign of injury (; Ling et al., 2009; Phillips & Richmond, 1991). Exposure to blast affects the whole body, especially the air-filled organs and those surrounded by fluid, including the brain and spinal cord (Elsayed, 1997; Mayorga, 1997). These complex injury mechanisms may interact and result in greater impairment or prolong the recovery period according to the Defense Veterans Brain Injury Center (2011). To better understand the effects of bTBI, this experiment used an animal model of blast over pressure (BOP) to induce a bTBI and measured the subsequent effects on neurological functioning, anxiety-like and depressive-like behaviors.

Stress

The relationship between bTBI and psychological stress is not well characterized (Hoge et al., 2008; Vasterling et al., 2006). Stress is defined as a "process by which environmental events threaten or challenge an organism's well being and how the organism responds to the threat" (Baum, Gatchel, & Krantz 1997, p. 61). Stress has been conceptualized as an important mediator of the health-behavior relationship and a common and inevitable aspect of life that has pervasive influence on bodily systems. Stress modifies the impact of insult or pathogens on health and health behaviors (Baum & Singer, 1987). The study of stress and illness can be traced from Hippocrates, who described the process of disease as separate from the "toil" of dealing with that disease, to

the 20th century where the physiology of the stress response was characterized by Cannon (1935), Selye (1946), Mason (1975) and others. The work of Selye (1946) on the General Adaptation Syndrome revealed the role of stress hormones and the effects of chronic stress on function, specifically how the system could be exhausted with repeated or chronic activation by non-specific stressors. Another concept that Selye (1976) brought to the study of stress was that the stimuli for the stress response are not just non-specific, but could carry a positive or negative valence and produce the same physiological response. Stimuli with a positive valence are called eustress (e.g., getting married) and stimuli with a negative valence are called distress (e.g., getting fired). The physiological response to stress is important, but that response can be mediated by how the stressor is perceived. Lazarus (1966) and others, including Mason (1975), emphasized the importance of the cognitive appraisal of the stressor and coping mechanisms in the stress response. Stress also can be experienced in anticipation of the stressor or after the stressor has passed (i.e., as an after-effect; Glass & Singer, 1972). The stress response can happen acutely in an adaptive way that allows the organism to respond to the threat. It can be a repeated exposure with multiple exposures to the threat separated in time, called repeated-acute (e.g., driving in traffic). Or the stress can be experienced as a chronic, unsubsiding exposure to the threat (e.g., chronic pain).

The present work is concerned with "Warrior Stress," or the unique experiences of service members who are deployed, injured, and may have a TBI and/or mental health problems as a result. Warriors experience both eustress and distress. They have stress in anticipation of a threat (e.g., when preparing for a mission). They respond acutely to stressful situations (e.g., seek cover when hearing alarms for incoming fire), have

repeated-acute exposure to stressors (e.g., noise from weapons, generators, artillery), and experience episodic and chronic stress while deployed (e.g., being away from home and loved ones). Psychological stress is an important factor in the lives of service members and was included in this experiment because of its potential impact on effects of and recovery from TBI.

Warrior Stress. Warriors experience unique stressors associated with deployment and combat exposure. The 2011 Joint-Mental Health Advisory Team (J-MHAT), based on surveys from approximately 1,000 deployed Army Soldiers and Marines, indicates more combat exposure and rates of anxiety, depression, and acute stress disorder (ASD) compared to each of the reports issued between 2003 and 2010. Some deployment stressors include: threat of death, variations in psychological and physiological arousal levels, sleep deprivation, physical injury, and environmental exposures. Unique deployment stressors place Warriors at significant risk of physical and mental health consequences that disrupt day-to-day life as well as performance under pressure (Vasterling et al., 2006). These consequences include neurological compromise (sensory and motor problems, problems with sustained attention, and problems with learning; Vasterling, et al., 2006) and anxiety, depression, and post traumatic stress disorder (Hoge et al., 2004). Hoge and colleagues (2004) reported that a marked percentage of service members met criteria for major depression, PTSD, and alcohol misuse after deployment based on anonymous surveys collected before and after deployment to Iraq and Afghanistan. In another sample, from a population-based descriptive study of all Army Soldiers and Marines who completed the routine post-deployment health assessment (PDHA) between 2003 and 2004, 35% of Veterans who

served in Iraq sought mental health services in the year following their return and 12% were diagnosed with a mental health problem (Hoge, Auchterlonie, & Milliken, 2006). Additionally, multiple deployments have been associated with more psychological problems, decreased morale, and increased use of medicartions to treat mental health problems, combat stress, and sleep problems (JMHAT, 2011).

Given the co-occurrence of bTBI and psychological stress, it is important to determine if bTBI and psychological stress interact. This experiment investigated the effects of both variables alone and in combination to test for possible interactions. The following section describes some of the literature available on the association between bTBI and mental health problems.

bTBI and Mental Health

In addition to the physical symptoms, TBI patients may experience mental health problems. Persistent mental health problems are like invisible wounds for TBI patients that disable them personally, socially, and occupationally (Rao & Lykesos, 2000). Mental health complications following TBI could manifest in depression, anxiety, PTSD, and substance abuse. This experiment measured anxiety-like and depressive-like behaviors.

TBI is associated with major depression (Hoge et al., 2008; Jorge et al., 2004; Rao & Lyketsos, 2000; van Reekum, Cohen, & Wong, 2000). Jorge and colleagues have been studying psychiatric disorders following brain injury for decades (e.g., Jorge, Robinson, & Arndt, 1993; Jorge & Robinson, 2002; Jorge et al, 2004). In two separate samples of TBI patients, ranging from mild to severe, injured in motor vehicle accidents, nearly 50% of the patients with TBI developed major depressive disorder (Jorge & Robinson, 1993)

or a mood disorder (Jorge & Robinson, 2002) in the year following injury. According to Jorge et al. (2004), major depression following a TBI can contribute to cognitive processing dysfunction, negative affect, and anxiety. Anxiety disorders also are common (11-70% of patients) following a TBI (Jorge & Robinson, 2002; Jorge et al., 2004; Klonoff, 1971; Lewis & Rosenberg, 1990; Rao & Lyketsos, 2000; van Reekum, Cohen, & Wong, 2000).

Stress can manifest in other disorders such as ASD and PTSD. Returning Warriors also are affected by these disorders and the associations between TBI and ASD and the lasting effects of PTSD is unclear (Martin et al., 2008). Mild TBI with loss of consciousness is strongly associated with PTSD (Hoge et al., 2008). bTBI and PTSD have many overlapping symptoms such as: difficulty concentrating, sleep disturbance, and mood alteration which contribute to the uncertainty surrounding these conditions and may lead to misdiagnosis (Ling et al., 2009). Misdiagnosis could result in improper, possibly insufficient treatment for the service member (Ling et al., 2009). It also is possible for TBI and PTSD to be comorbid.

It is important to determine the effects of bTBI and stress and how each variable is contributing to or altering the other with regard to behavioral proxies of mental health outcomes. This experiment examined these variables and used behavioral measure of anxiety-like and depression-like behaviors to determine these effects. Individual differences also may be contributing to the effects of bTBI and its association with mental health problems. The most basic individual difference is sex. The next section discusses the rationale for the inclusion of this variable.

bTBI and Sex Differences

Male and female Warriors and civilians are exposed to blasts from explosions and psychological stress. The male psychological and biological stress responses have been extensively studied in animals and humans for decades and can be summarized as the "fight-or-flight response" (Cannon, 1935; Faraday, 2005; Mason, 1975; Selye, 1946; Selye, 1973). In situations of stress, males are more likely to become aggressive (fight) or to escape the threatening situation (flight). Escape behavior was measured in the present experiment. In addition, anxiety-like behavior was measured (see Dependent Variables in the Methods section). The female stress response may be different from the male response and some have argued that it should be called the "tend-and-befriend" response (Taylor, Klein, Lewis, Gruenewald, Gurung, & Updegraff, 2000). When a female experiences stress, she will increase care for her young and/or seek social interaction. Therefore, it is expected that the response to stress will differ for males and females. Recent reports, regarding individuals hospitalized while deployed, show that female service members have a 70% decreased risk of TBI admission (Wojcik et al., 2010). This decreased incidence may be related to behavioral factors (e.g., decreased exposure to combat, better use of protective equipment) but it may also be because of biologically protective mechanisms. This experiment used male and female rats to test if bTBI and stress affected males and females differently.

bTBI and Time

According to the *Traumatic Brain Injury Care in the Department of Defense* report in 2009, the short and long term effects of blast injury are still unknown (Defense Centers of Excellence [DCoE] for Psychological Health and Traumatic Brain Injury and

the Defense Veterans Brain Injury Center [DVBIC], 2009). Common symptoms reported following a TBI are: headache, vertigo, memory deficits, irritability, attention deficits, and sleep difficulties (Ling et al., 2009; Martin et al., 2008). These symptoms can interfere with a person's daily routine and personal relationships (Martin et al., 2008). TBI with loss of consciousness places Warriors at increased risk of health problems, including poorer general health, missed workdays, increased medical visits, and increased somatic and post-concussive symptoms (Hoge et al., 2008). According to the Department of Defense TBI Surveillance Database, 85-90% of Troops with TBI from combat report a decrease in symptoms in the days to weeks following injury (DCoE & DVBIC, 2009). In another report, 20-40% of Veterans with mild TBI had lasting symptoms upon return from deployment (U.S. Department of Defense and Department of Veterans Affairs, Traumatic Brain Injury Task Force, 2008). Additionally, unrecognizable or subtle symptoms following a bTBI may go unnoticed by a service member and delay treatment (Ling et al., 2009). To begin to investigate the acute versus longer lasting effects of bTBI, this experiment examined responses at two time points: 1 day after injury and 8 days after injury.

The Value of Animal Models

Based on the information available from human patients, it is evident that bTBI, stress, and mental health problems are associated. The studies from which these associations come are correlational, lack experimental controls, and therefore cannot prove causality. A clear directional or causal link between the variables has not been identified because a true experiment of blast in humans is not possible. Therefore, the best possible experimental investigation of blast needs to be done using an animal model. Animal models are more ethical and more logistically feasible than human experiments

of brain injury. A true experimental investigation, using an animal model of bTBI, allows for a clear segregation of some key variables involved: the injury alone; the stress associated with deployment and injury; the sex of the patient; the time course of the variables; and control of other relevant biological (e.g., genetics) and environmental variables (e.g., temperature, humidity, sounds, odors).

Animal modeling allows for ethical and controlled experimental investigations of both biological and psychological variables. Human-animal comparisons have been made since the Darwinian revolution of the 19th century, and over time the approach has evolved from purely behavioral to more cognitive investigations (Davey, 1983). Rats were domesticated for, bred, and have been used in scientific research to model the human condition for over 160 years (Jacob, 1999). Additionally, rats are considered the primary species (other than humans) to use in laboratory investigations of behavior (Whishaw & Kolb, 2005) based on the logistical feasibility and the parallel findings to human conditions. For the last 30 years, the Grunberg laboratory has used rats to model human psychological conditions (Faraday & Grunberg, 1999; Winders & Grunberg, 1989) including an investigation of brain injury (Elliot, 2004). The present experiment was part of a new series of experiments that were designed to examine the effects of brain injury on behavior. Therefore, a great deal of consideration went into selecting the appropriate research paradigm to use. The following section provides an overview of animal models of brain injury that were considered.

Animal models of brain injury. Animal models of TBI include direct impact and indirect damage methods. Direct impact methods are valid models for the pathology and physiology of the human condition and reliably test interventions and treatments, but

they may not be appropriate for studying human brain injury caused primarily by blast from improvised explosive devices (IEDs). Nevertheless, these models are currently being used to investigate TBI. Three of the most common direct impact methods used to test the effects of injury on behavior are briefly discussed below. This information is presented for use when comparing the results of other studies to the present work. Three methods commonly used in laboratory experiments are fluid percussion, controlled cortical impact, and weight drop. The benefits and limitations of these models are discussed.

Fluid percussion. The fluid percussion model replicates contusion without skull fracture. The impact is delivered in this model using a fluid pressure pulse to the dura (Ling, Lee, & Kalehua, 2004; McIntosh, Noble, Andrews, & Faden, 1987; Schmidt & Grady, 1993). The fluid percussion model has restricted biomechanical control and limited validity to the human condition because at higher levels it causes too much damage (e.g., disproportional brainstem involvement and neurogenic pulmonary edema; Cernak, 2005). This model's limitations lead to the development of the controlled cortical impact model.

Controlled cortical impact. The controlled cortical impact (CCI) model was developed to allow for better control over biomechanical factors (e.g., velocity of impact and depth of deformation) than that offered by the fluid percussion model. The impact for this model is delivered using a compressed air-driven metallic piston to the dura (Cherian, Robertson, Contant, & Bryan, 1994; Dixon, Clifton, Lighthall, Yaghmai & Hayes, 1991). This model is used to replicate human brain injury with skull deformation and related cortical compression (Cernak, 2005). This model produces a focal injury

with limited use for studying diffuse injury (e.g., diffuse axonal injury). Because of this limitation, the weight drop model was developed.

Weight drop model. An impact model that can produce more diffuse injury is the weight drop model. The weight drop model (also called Marmarou's weight drop model; Marmarou et al., 1994; Foda & Marmarou, 1994) is frequently used to model constrained impact acceleration head injury. The impact for this model is delivered by a column of brass weights that are dropped from a designated height falling freely by gravity through a Plexiglas tube. A stainless steel disc is fixed to the animal's exposed skull with dental cement. This model is strictly controlled, inexpensive, easy to perform, and reliably produces graded diffuse axonal injury (Cernak, 2005). The limitation of this method, with regard to the present experiment, is that it requires contact with the brain tissue. In bTBI cases it is possible that injury occurs without any contact with the head or brain tissue. For this reason, it is better to use indirect models to manipulate injury in investigations of the effects of bTBI on behavior.

Blast models. Shock or blast waves are used to create indirect damage. Blast injury is more diffuse and may or may not include impact. Unlike direct impact injuries, the primary pressure from a blast wave alone can initiate functional, biochemical, and morphological changes in the brain (Cernak et al., 1996), but it is not clear how the primary blast wave injures the brain (Chavko et al., 2011). Experiments using indirect damage models (e.g., blast over pressure model) have shown the brain to be susceptible to damage, including: significant blood brain barrier breakdown (Readnower et al., 2010); transient cognitive dysfunction (Long et al., 2009); increased intracranial pressure (Saljo, Svensson, Mayorga, Hamberger, & Bolouri, 2009); and brain edema (Beaumont et

al., 2000). In experiments of indirect damage, the shock or blast waves have been generated by explosives (Axelsson et al., 2000; Bauman et al., 2009; Cernak et al., 1990, 1991; Richmond et al., 1967; Savic et al., 1991) and compressed air (Bogo, Hutton, & Bruner, 1971; Bauman et al., 1997; Cernak et al., 1996b, 2001a, 2001b; Chavko et al., 2007; Chavko et al., 2011; Jaffin et al., 1987; Long et al., 2009b; Moochhala, Md, Lu, Teng, & Greengrass, 2004; Mundie, Dodd, Lagutchik, Morris, & Martin, 2009; Readnower et al., 2010; Saljo, Bao, Haglid, & Hansson, 2000; Saljo, Bao, Hamberger, Haglid, & Hansson, 2001; Saljo et al., 2009). In these models various animals have been used including: monkeys (Bogo et al., 1971), goats (Damon et al., 1968; Richmond et al., 1967), pigs (Axelsson et al., 2000; Bauman et al., 2009), sheep (Cernak et al., 1990, 1991; Mundie et al., 2000; Savic et al., 1991), and rodents (Baumann et al., 1997; Cernak et al., 1996b, 2001a, 2001b, Chavko et al., 2007; Chavko et al., 2011; Long et al., 2009b; Moochhala et al., 2004; Readnower et al., 2010; Salijo et al., 2000, 2001, 2009).

A gold standard model and species for investigating TBI experimentally has yet to be identified. The blast over pressure model (BOP) in rats has been used for decades to investigate other forms of injury (e.g., ear, lungs, intestine) and has recently been employed to investigate brain damage with no visible head injury (Cernak, 2005). In the last five years, this model has been used to investigate mechanisms underlying brain damage from blast and to break down the complex injury from clinical and experimental perspectives (Cernak & Noble-Haeusslein, 2010; Cernak, 2005; Kazanis, 2005; LaPlaca, Simon, Prado, & Cullen, 2007; Manvelyan, 2006; Morales et al., 2005; Potts et al., 2009; Weber, 2007). Despite this work, there is little known about the effects of this model on behavior. For this reason, the BOP model was selected to be used in this

experiment to model the human exposure to blast and measure effects on behavior. Using this model the goal was to define a behavioral phenotype of bTBI and test for alterations in the effects of blast by psychological stress, sex, and time. The following section presents the specific aims and hypotheses of this experiment.

Overview and Specific Aims

The present experiment was designed to determine the effects of bTBI and psychological stress on behavior responses of male and female rats. There were four specific aims: (1) to determine the effects of blast on neurobehavioral functioning and mental health-like behaviors; (2) to determine the effects of stress alone and stress in combination with blast on neurobehavioral functioning, and mental health-like behaviors; (3) to examine if blast and stress have different effects on neurological functioning and mental-health like behaviors in males and females; (4) to determine if time is important for the measurement of neurobehavioral functioning and mental-health like behaviors following blast.

Hypotheses

There were several hypotheses associated with each specific aim.

Specific Aim 1: To determine the effects of blast on neurobehavioral functioning and mental health-like behaviors.

Hypothesis 1a. Blast will deleteriously affect neurobehavioral functioning that will be detected by higher neurological severity scores.

***Hypothesis 1b*.** Blast will deleteriously affect mental health-like behaviors (including anxiety-like and depression-like behaviors) that will be detected by decreased center time and vertical activity for blasted animals.

Rationale. Neurological dysfunction following TBI is detectable in humans using instruments like the Glascow Coma Scale. Other symptoms following injury can be accessed using the Neurobehavioral Symptom Inventory (NSI; Cicerone & Kalmar, 1995) and self-report measures such as the Post Concussive Symptoms Scale (Chen,

Johnston, Collie, McCrory, & Ptito, 2007). The NSI is currently used to access symptoms in Veterans injured during deployment by the Veteran's Administration and the Department of Defense (DVBIC, 2011). TBI also has been correlated with other symptoms, including: chronic pain (Nampiaparampil, 2008); migraine headaches (Patil et al., 2011) and auditory, visual, or dual sensory impairment (Lew et al., 2011). Clinically, TBI is also associated with mental health problems (Hoge et al., 2008; Jorge & Robinson, 2002; Jorge et al., 2004; Klonoff, 1971; Lewis A, 1942; Lewis & Rosenberg, 1990; Ling et al., 2009; Rao & Lyketsos, 2000; van Reekum, Cohen, & Wong, 2000). Ongoing animal studies using the BOP model of TBI have reported deleterious physiological effects of the blast (Cernak, 2011, 2005; Chavko et al., 2010; Readnower et al., 2011). Additionally, investigators have reported disruptions in neurobehavioral performance (Long, Bentley, Wessner, Cerone, Sweeney, & Bauman, 2009) and changes in mental health-like behaviors (Kovesdi et al., 2011; Kwon et al., 2011) following exposure to blast in rats.

Specific Aim 2: To determine the effects of stress alone and stress in combination with blast on neurobehavioral functioning and mental health-like behaviors.

Hypothesis 2a. Stress will deleteriously affect neurological functioning that will be detected by higher NSS-R scores.

Hypothesis 2b. Stress will deleteriously affect mental health-like behaviors (including anxiety-like and depression-like behaviors) that will be detected by decreased center time and vertical activity for stressed animals.

Hypothesis 2c. Stress will either exacerbate or attenuate the effects of blast on neurobehavioral functioning and mental-health like behaviors.

Rationale. Behavioral changes in response to stress have been reported for years using rodent models of stress (e.g., Faraday, Blakeman, & Grunberg, 2005; Faraday, O'Donoghue, & Grunberg, 2003; Selye, 1936; Weiss, 1972). The interaction of blast and stress (exacerbate or attenuate) is hypothesized based on the classic work of Yerkes and Dodson (1908). Performance is related to arousal following an inverted U-shaped function (i.e., low amount of arousal produces limited performance; a moderate amount of arousal is necessary for optimal performance; a high level of arousal results in decline in function). Based on this relationship, stress either will exacerbate the effects of blast or attenuate the effects of blast depending on the level of arousal that is created by the variables alone and in combination, and the rats' response to the arousal.

Specific Aim 3: To examine sex as an individual difference variable that may change responses to blast and stress and the effects the two variables have on neurobehavioral functioning and mental-health like behaviors.

Hypothesis 3a. Blast will affect males and females differently, detected using measures of neurobehavioral functioning and mental health-like behaviors.

Hypothesis 3b. Stress will affect males and females differently, detected using measures of neurobehavioral functioning and mental-health like behaviors.

Hypothesis 3c. Blast and stress in combination will affect males and females differently, detected using measures of neurobehavioral functioning and mental-health like behaviors.

Rationale. There are currently no experimental investigations that directly compare the effects of blast and stress on male and females rats. However, as previously discussed, males and females have different biological and psychological responses to

stress (Cannon, 1935; Taylor et al., 2000). This differential response may play a role in the effects of blast and stress on neurological functioning and mental health-like behaviors.

Specific Aim 4: To determine if time since injury is important for the measurement of neurobehavioral functioning and mental-health like behaviors following blast.

Hypothesis 4. Measurements of neurobehavioral functioning and mental-health like behaviors taken 1 day following blast will be different from measurements taken 8 days after blast exposure.

Rationale. Reports from human TBI literature differ on the time course for symptoms following injury. Some reports indicate that self-reported symptoms worsen over time (e.g., Belanger et al., 2011; Emanuelson, Andersson Holmkvist, Bjorklund, & Stalhammar, 2003; Montgomery, Fenton, McClelland, MacFlynn, & Rutherford, 1991), whereas others (i.e., objective indicators of function, such as cognitive or behavioral measures) report that symptoms are worse acutely and dissipate over time (e.g., DoD TBI Surveillance Database, 2009). Most of the current animal investigations of blast on behavior include only one time point measure of effects (e.g., 24 hours up to 11 days post injury; Chavko et al., 2011; Kamnaksh et al., 2011; Long et al., 2009; Readnower et al., 2011). There are a few investigators that examine blast and other variables (e.g., environmental enrichment; sham blast injuries; Kovesdi et al., 2011; Kwon et al., 2011) or use other injury methods (e.g., CCI, fluid percussion) over longer periods of time that can be used to make hypotheses about the present experiment. Some of these investigators have reported persistent neurological and behavioral effects 14 days post

injury using CCI (Cole et al., 2011) and persistent anxiety-like behavior using fluid percussion (Jones et al., 2008).

Methods

To address the above hypotheses, this experiment was conducted as a 2 (no blast, blast) x 2 (no stress, stress) x 2 (male, female) x 2 (1day, 8 days post injury) full factorial mixed design. This experimental design results in 16 experimental conditions. There were eight or twelve subjects in each condition (Table 1). The number of subjects per condition was determined based on many years of animal experiments in the Grunberg Laboratory evaluating a variety of stressors and other independent and dependent variables similar to the variables examined in the present investigation (Acri, Grunberg, & Morse, 1991; Faraday, O'Donoghue, & Grunberg, 2003; Grunberg & Bowen, 1985; Grunberg & Faraday, 2000; Winders & Grunberg, 1989). Additional subjects were run in male, time point one conditions for use in another experiment, so these cells have 12 subjects. Conditions were counterbalanced over seven cohorts for this experiment to ensure that historical and environmental factors would not confound experimental outcomes. Animal husbandry conditions, independent variables, dependent variables, experimental timeline, data analytic strategy, and power analyses are explained in detail below.

Animals and Housing

The subjects were 79 male and 64 female Sprague-Dawley (SD) rats from Charles River Laboratories (Wilmington, Massachusetts). Rats were approximately 54 days old upon arrival. This age was selected to model age demographics of Warriors. Investigators have determined that rat adolescence ends around 42 and 55 days for female and male rats, respectively (Ojeda & Urbanski, 1994; Spear & Brake, 1983). Adulthood is considered to begin around 60 days (Faraday, Elliot, & Grunberg, 2001). The rats used in this experiment (54 days to 71 or 78 days) are comparable in age to young adult

Warriors who have deployed in support of Operations Iraqi and Enduring Freedom. (OIF/OEF). The SD strain was selected because SD rats are extensively used in stress and brain injury research (Chavko et al., 2011; Faraday, Blakeman, & Grunberg, 2005; Faraday, O'Donoghue, & Grunberg, 1999; Porterfield et al., 2011; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Readnower et al., 2010; Shaham, Klein, Alvares, & Grunberg, 1993). Sex was one of the independent variables of this study, so male and female rats were included.

Rats were individually housed in standard polycarbonate shoebox cages (42.5 x 20.5 x 20 cm) with hardwood chip bedding (Pine-Dri). Subjects were individually housed because social and environmental enrichment can have effects on behavioral and biological variables (Elliot, 2004; Rosenzweig & Bennett, 1996; Rosenweig, Krech, Bennett, & Diamond, 1962; Woodcock & Richardson, 2000) and improve recovery following brain injury (Elliott & Grunberg, 2005). Cages were changed twice a week by Laboratory Animal Medicine (LAM) husbandry staff to ensure that rats did not experience additional stress from excessively soiled housing conditions. Subjects had continuous access to standard, bland, laboratory chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at 23°C with 40% relative humidity on a 12 hr reverse light cycle (0600-1800 lights off). To measure rats during their active time, a reverse light cycle was used. Rats are nocturnal animals, with normal high-activity during dark periods. The experiment was conducted under a protocol approved by USUHS Institutional Animal Care and Use Committee (MPS-09-732; Biobehavioral assessments of traumatic brain injury in rats) and conducted in full compliance with the National

Institutes of Health Guide for Care and Use of Laboratory Animals (Committee for the update of the guide for the care and use of laboratory animals, 2011).

Independent Variables

There were four independent variables in this study: injury (no blast, blast); stress (no stress, stress); sex (male, female); time point (1 day, 8 days post injury).

Blast overpressure (BOP). The BOP method of blast exposure resembles the conditions to which Troops are exposed from IED blast on the battlefield (Long et al., 2009). This method can produce different air-blast levels that are survivable and can be used to inflict injury to be studied. The air-blast exposure of 126 kPa was used in the present experiment to induce a mild to moderate injury to model human mild TBI from blast exposure. Long and colleagues (2009) measured neuropathology following a 126 kPa blast and two other levels (114 and 147 kPa) in rats. The investigators reported that 126 kPa produced a diffuse injury, whereas lower levels showed no neuropathological changes and 147 kPa produced significant cell loss and other pathology (Long et al., 2009). Mild TBI is associated with diffuse axonal injury in humans (Arfanakis et al., 2002; Mittl et al., 1994). Therefore, the BOP model, at 126 kPa of exposure should produce a similar mild injury in the rat model.

Rats in the bTBI experimental groups were transported to Walter Reed Army Institute of Research/Naval Medical Research Center (WRAIR/NMRC), Forest Glen and back to USUHS in a closed van provided by USUHS. Rats were housed in cardboard transportation cages. Cages were secured from sliding and room temperature was maintained while being transferred from the van to the BOP laboratory. Rats are transferred to a holding area near the BOP laboratory, but separated by a cinderblock wall

and sound attenuating wall. Immediately prior to the blast exposure, rats were individually placed into a polystyrene container and anesthetized (5% isofluorane mixed with oxygen at 1L/min for 2 min) before exposure to BOP (Chavko, Koller, Prusaczyk, & McCarron, 2007). Each anesthetized rat was placed into a metal, mesh basket and secured with two rubber straps (2.54 cm). Rats' bodies were oriented facing the origin of the blast. This orientation provides frontal exposure which results in higher amplitudes and longer durations of the pressure waves in the brain compared to side-on orientation (Chavko et al., 2011). The basket was then placed at the mouth of the blast tube. The blast tube is a horizontally mounted, 12-inch diameter, circular, 19.5 ft long, steel tube (Figure 1). The tube is divided into a 2.5 ft compression chamber separated from a 17 ft, expansion chamber by polyethylene MylarTM sheets (0.0254 cm thick; Du Pont Co., Wilmington, DE). Pressure increases in the compression chamber until the Mylar sheet ruptures and generates a pressure wave that produces an exposure pressure of 126 kPa for less than a second.

After injury the rats were returned to their transport cages, monitored until consciousness was regained (approximately 5 minutes), transported back to the USUHS LAM housing facility, and returned to their home cages. Rats in the non-injured experimental conditions remained in the animal housing rooms at USUHS.

Stress manipulation. In the combat environment, uniformed service members are exposed to stressful, life-threatening and unpredictable situations (Joint Mental Health Advisory Team 7, 2011). These conditions may result in deleterious health consequences including anxiety, depression, substance abuse (Hoge, Auchterlonie, & Milliken, 2006, Hoge et al., 2004; Hoge et al., 2008; Tanielian, et al., 2008). To

determine psychological and biological effects of these negative health consequences, mechanisms underlying these relationships, and attenuation or prevention of these effects, it is valuable to conduct controlled experiments with animal subjects.

The Warrior Stress Paradigm (WSP) for rats is designed to model the anticipatory stress commonly experienced by Warriors as they prepare for and engage in life-threatening missions in hostile, deployed settings (Yarnell, Chwa, Hamilton, & Grunberg, 2011). This manipulation uses a combination of a predator stressor and unpredictable environmental stimuli. This model is based on previous studies, conducted in the Grunberg laboratory (Berger, 2009; Hamilton, 2010; Long, 2010; Perry, 2009; Starosciak, 2010;) and based on other reports (e.g., Hayley, Borowski, Merali, & Anisman, 2001; Willner, Towell, Sampson, Sophokleous, & Muscat, 1987).

Predator stress is a non-painful, but effective stressor used in rodent models investigating the effects of stress. Predator stress can be manipulated by presenting the actual predator or the odors of the predator. Exposure to predator stress produces increases in stress hormones (Berger, 2009; Campbell, Lin, DeVries, & Lambert, 2003; Hayley, Borowski, Merali, & Anisman, 2001). Exposure to predator stress also produces behavioral changes in rodents including differences in food consumption, anxiety-like behavior, startle response, freezing behavior, withdrawal behavior, and exploratory behavior (Belzung, El Hage, Moindrot, & Griebel, 2001; Berger, 2009; Endres, Apfelbach, & Fendt, 2005; Hamilton, 2010; Long, 2010; Masini, Sauer, & Campeau, 2005; Mechiel Korte & De Boer, 2003; Perry, 2009; Rose, 2011; Starosciak, 2010; Takahashi, Nakashima, Hong, & Watanabe, 2005). Predator stress was presented by

introducing a cotton ball with commercially available, synthetic fox urine (Buck Stop, Stanton, MI) into a test cage with the rat subject.

To model environmental stress experienced by Warriors and to avoid habituation to repeated presentation of the fox urine, unpredictable, non-painful stressors are included in the WSP. Unpredictable, non-painful stressors include noise, flashing light, and cage shaking. Unpredictable stressors are included because it is a face-valid model of human stress and also reliably produces alterations in stress hormones (Fride, Dan, Feldon, Halevy, & Weinstock, 1986; Weinstock, Matlina, Maor, Rosen, & McEwen, 1992), and behavior (Berger, 2009; Fride et al., 1986; Gonzalez Jatuff, Berastegui, Rodriguez, & Rodriguez Echandia, 1999; Hamilton, 2010; Long, 2010; Perry, 2009; Rose, 2011; Starosciak, 2010) in rodent studies.

Each animal, in the stress condition, was transferred from its home cage to the "stress cage" (29 x 18 x 12 cm) with a lid and no bedding (Figure 2). The fox urine (15mL) was absorbed by a large cotton ball and placed in varying spots in the stress cage. The procedure was conducted in a room separate from the housing room and the behavioral rooms. A bright, florescent, overhead light remained on during the procedure. The stress procedure lasted for 20 minutes. The stressors were administered for seven consecutive days in a manner designed to minimize habituation of the stress response. Figure 3 presents the WSP timeline. Animals in the non-stressed conditions remained in the housing rooms during stress manipulations.

Sex. Approximately 97% of mTBIs from OIF/OEF occur in males (Wojcik, Stein, Bagg, Humphrey, Orosco, 2010). In the civilian population, young men are two to three times more likely to sustain a TBI than young women (CDC, 2010). Most animal

studies of TBI use male rats. There are no studies that investigate how sex differences may inform the clinical sequellae following a TBI. This experiment examined behavioral differences in males and females following blast exposure and stress. It is important to determine if there are any sex difference in behavior following exposure to blast and stress because: (1) male and female civilians are exposed to bTBI in war zones; (2) information from this study may extrapolate to males and females suffering from other severities and types of traumatic brain injuries; and (3) any sex differences might suggest novel treatment strategies for brain injury.

Time. Time was investigated as a between-subjects variable in this experiment. Two different time frames were included to determine whether any effects of bTBI: (1) occurred shortly after injury and then resolved; (2) occurred shortly after injury persisted; or (3) increased over time. Rats were measured one day after injury or eight days after injury. The temporal pattern of response might suggest underlying biological mechanisms that could be investigated to inform prevention or treatment.

Dependent Variables

The dependant variables were a measure of neurological functioning and mental health-like behaviors. Neurological functioning was measured using the revised neurological severity scale (NSS-R). The mental health-like behaviors were assessed using activity measured in an open-field (OFA). The rationale for including these measures and a detailed description of the equipment and procedures used in this experiment are provided in this section.

NSS-R. The Revised Neurological Severity Scale (NSS-R) is a specific, continuous sequence of behavioral tests and observations (Grunberg, Yarnell, Chwa,

Hutchison, & Barry, 2011). This measure was originally designed to model a clinical neurological exam conducted in human neurology patients. This revised version of the animal model is based on several previous reports and has been modified to increase standardization. The NSS-R is based on the Neurological Severity Score (NSS). The NSS is a battery of motor and reflex tests used to assess the extent of brain injury in experimental rodents (Hamm, 2001; Mahmood et al., 2001; Shohami, Novikov, & Bass, 1995). The tests assess reflex suppression, general movement, and postural adjustments in response to a challenge. The NSS includes observations of behaviors and performance measures. NSS-R tasks 2, 3, and 10 are based on the methods of Shohami et al. (1995); tasks 1, 3, 5, 6, 8, and 10 are based on the methods of Mahmood et al. (2001); tasks 6 and 10 are based on methods described by Hamm (2001); task 4 is based on the methods of Marti et al. (2005); tasks 7 and 9 are new. It is unclear from the previous versions of this measure the order in which the tests should be conducted. The NSS-R specifies the order in which the test should be conducted. The tests are ordered to ensure that subsequent measures are not affected by the preceding measure. In the previous version of the NSS, behavior was scored in a binary fashion with the absence of behavior assigned one point. Higher total scores indicate a greater extent of injury. The Shohami scale has a series of behavioral tasks that are each scored in a binary fashion (where 0= normal response; 1= failure to perform task). The Mahmood scale similarly uses a binary scale (where 0= normal response; 1= failure to perform task) to evaluate performance on a series of behavioral tasks. By contrast, the NSS-R uses a three-point Likert scale, in which a normal, healthy response is assigned a “0,” a partial or compromised response is assigned a “1,” and the absence of a response is assigned a “2.” This three-point scale is clear and

reliable. The NSS-R has a scoring range of 0-20, which is designed to increase sensitivity. Higher scores reflect greater extent of injury. The NSS-R was scored by a trained rater. Scores were totaled and the average between the two raters was used as the final NSS-R score. Figure 4a provides an example of the score sheet used to make these assessments.

The testing was conducted using two empty "guinea pig" polycarbonate cages (46 cm × 36 cm × 20 cm) with no bedding or lid (Figure 4b). The larger cage was used for easier handling of the animal and adequate viewing room for the rater. There were two NSS-Rs conducted in this experiment as a within-subject measure: one before injury (baseline) and one after injury (post injury). It was important to collect each rat's baseline neurological behavior score to be compared to its post injury score, because this measure may be prone to individual variation. A change score was created by subtracting the baseline score from the post injury score and was used for data analysis. Details for each measure are listed below. Each task may be repeated up to three times if necessary (e.g., the observer missed the response, there was no response to the first stimulus). In these cases the final score given by the rater is based on the "best" performance. The ten individual tasks of the NSS-R are described briefly below.

General balance test. The rat's ability to walk on a balance beam is assessed first, as a measure of gross motor balance. The balance beam (2 cm wide x 100 cm long) is placed lengthwise at a height of 29 cm above the test cage by placing the ends of the balance beam on top of two pairs of stacked cages. The rat is gently placed onto the balance beam and observed. The rat's movement on the balance beam is scored as 0, 1, or 2. A score of 0 is recorded if the rat maintains balance and walks successfully on the

beam. A score of 1 is recorded if the rat balances on the beam but does not walk along the beam. A score of 2 is recorded if the rat does not balance or walk on the beam at all. The second test begins immediately after test #1.

Landing test. In this test, each rat is dropped from a height of 29 cm above the test cage floor. As each rat lands in the cage the reflexes of its paws and body posture are observed and rated. A score of 0 is recorded if the rat shows normal reflexes when landing. A score of 1 is recorded if the rat shows partial reflexes that are compromised in some way. A score of 2 is recorded if the rat does not use landing reflexes when landing and instead falls flat onto the cage floor. The third test begins immediately after test #2.

Tail raise test. Immediately following the landing test, the rat is gently lifted by the base of the tail (dorsal side up) to a height of approximately 50 cm above the cage floor. When the rat is lifted, the reflexes of its forelimbs and hindlimbs are observed and scored for the proper flexion and extension. A score of 0 indicates normal reflexes, a score of 1 indicates partial reflexes, and a score of 2 indicates no reflexes are observed (the rat is limp when lifted by the tail). After the tail raise test, the rat is taken to the second cage, to conduct the drag test.

Drag test. While continuing to hold the rat by the base of the tail, the rat is placed on the floor of the second test cage (ventral side down), with the rat facing and close to one of the less wide walls. Allowing only the rat's forepaws to remain on the floor of the cage, the rat is gently dragged backwards at a constant speed (about 20 cm/sec) across the length of the cage. The rat's behavior, while being dragged, is observed. A score of 0 indicates extension of forepaws and effort to slow down the drag or to pull away from the

drag. A score of 1 indicates some effort to slow down the drag or to pull away. A score of 2 indicates no resistance.

Righting reflex. After the conclusion of the drag test, the rat remains in the second test cage for the remainder of the reflex tests. The rat is placed on its back and observed. A score of 0 indicates that the rat gets onto four paws without difficulty. A score of 1 indicates a partial "righting" or complete righting with difficulty. A score of 2 indicates an inability to right completely.

Ear reflex. The ear reflex is tested by lightly touching the auditory meatus with a long (approximately 10 cm) Q-tip and observing responses. A score of 0 indicates a flattening of the ear flap or movement away from the stimulus. A score of 1 indicates partial response and a score of 2 indicates no response. The Experimenter holds the rat in his/her non-dominant hand (with tail secured using the pinky) using the dominant hand to direct the Q-tip. The Experimenter continues to hold the rat and proceeds to the next test, using the same Q-tip.

Eye reflex. The eye reflex is tested by lightly touching the eye with a Q-tip and observing the response. The response is scored according to the following criteria: 0 indicates a complete and immediate eye blink; 1 indicates a delayed eyeblink; and 2 indicates no response. The Experimenter places the rat back into the second test cage for the next test.

Sound reflex. This reflex is a movement in response to the noise of a short, sharp clap of the experimenter's gloved (latex) hands. The reflex is observed and rated as follows: 0 for a quick jumpy movement followed by freezing; 1 for a slow movement and/or no freezing; and 2 for no response. The Experimenter waits 10 seconds before

proceeding to the next test, to allow the rat time to react to the sound stimulus. It also has been found that touching the rat following the startle tests results in squeaking that may not be related to the pinch from the next test, so before applying the pinch the Experimenter should pet and reposition the rat.

Tail reflex. For this test the Experimenter secures the rat in place on the floor of the test cage with his/her non-dominant hand. This reflex is tested by applying a brief pinch using the experimenter's fingers (thumb and index finger) to the middle area of the rat's tail and observing its response. The response is scored as: 0 for a marked and immediate squeak; 1 for a delayed or weak squeak; and 2 for no response. In some instances the squeak, especially in male rats, is very muted and sounds more like a gasp for breath; this response should be scored as a 1. Another response that also can be scored as a 1 is a turn as if to bite the Experimenter. This response is typically seen in mice, but has been seen in rats and should be scored accordingly.

Paw flexion reflex. For this test the Experimenter secures the rat in place on the floor of the cage using his/her non-dominant hand and extends the right hind paw. The Experimenter then uses his/her thumb and index finger to apply a brief pinch to the space on the hind paw where the bones of the toes extend outward. This test is scored based on eliciting a withdrawal response. The test is scored as follows: 0 for a limb withdrawal reflex; 1 for a partial or delayed withdrawal reflex; and 2 for no response.

Open field activity. Locomotor activity (OFA) is a collection of sensitive, unconditioned behaviors that occur when an animal moves in its environment. These measures have been used in rat studies investigating various variables (stress, addiction, social enrichment, brain injury) in our laboratory for many years and provide reliable and

valuable data about gross motor movement and specific movements related to psychological conditions (e.g., anxiety-like and depressive-like behaviors; Bowen, Eury, & Grunberg, 1986; Elliott, Faraday, Philips & Grunberg, 2004; Elliot & Grunberg, 2005; Faraday, Elliott, Phillips, & Grunberg, 2003; Faraday, O'Donoghue, & Grunberg, 2003; Faraday, Scheufele, Rahman, & Grunberg, 1999; Grunberg & Bowen, 1985; Morse, Davis, Popke, Brown, O'Donoghue, & Grunberg, 1997).

Open field activity was measured in this experiment to provide information about gross motor movement, general health, depressive-like behavior, and anxiety-like behavior. OFA also measures general movement, a variable important when interpreting other behavioral measures (e.g., NSS-R) that require the animal to move. The three parameters of OFA used in the present experiment were horizontal activity, vertical activity, and center time. Horizontal activity (HA) provides information about gross motor performance and general health. Vertical activity (VA) provides an index of depression. Rats that exhibit vertical movement escape behaviors are showing less depression-like behaviors (Berger, 2009; Grippo, Beltz, & Johnson, 2003; Hamilton, 2010; Long, 2010; Perry, 2009; Sarkisova, Kulikov, Midzyanovskaya, & Folomkina, 2008; Shafer, 2006; Starosciak, 2010; Zhuang, Xu, & Chun-Zhi, 2007). Center time (CT) provides an index of anxiety. There is an inverse relationship between center time and anxiety.

OFA was measured using an Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM (16 TAO); Accuscan Electronics, Columbus, OH), located in a room constructed to minimize sound. Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas arena with a Plexiglas lid (Figure 5). The lid has multiple

holes to ensure that subjects have adequate ventilation but cannot escape during data collection. Sixteen paired photocell arrays measures horizontal locomotor activity and vertical activity. Data was automatically gathered and transmitted to a computer via an Omnitech Model DCM-I-BBU analyzer. Rats were measured for 1 hour during the dark cycle. Once subjects were placed in the test areas, the Experimenter turned off the lights and left the room. Chambers were cleaned between subjects with alcohol. OFA was measured twice in this experiment. The baseline measure was conducted one week prior to injury and the post injury measure was conducted one day or eight days after injury.

Experimental Timeline

On the day that rats arrived they were singly housed and randomly assigned to an experimental condition. Rats were then handled or "gentled" by the Experimenters for two days to get the animals used to being handled prior to beginning the experiments. Rats were then acclimated to the open field chambers for 1hour. Baseline OFA and NSS-R measures were conducted about a week after the rats arrived. The stress procedure began after baseline measures were complete and two days prior to blast injury. Rats also were stressed on the day of blast injury. There were no behavioral measures conducted on the blast injury day. Non-blasted rats remained in the housing rooms while blasted rats were transported to the blast facility. Stress continued for rats in 1-4 day (T1) conditions and post-injury OFA and NSS-R measures were conducted one day after injury. T1 rats were sacrificed four days after injury and brain and blood samples were collected for another experiment. Rats in the 8-11 day (T2) conditions remained in the housing room until the eighth day. On the eighth day stress resumed for the T2 stress rats and OFA and NSS-R measures were conducted for all remaining subjects. T2 rats were

sacrificed on day 11 and brain and blood samples were collected for another experiment. Figure 6 presents the experimental timeline.

Data Analytic Strategy

Repeated measures analysis of variance (RMANOVA) were conducted using all independent variables for each of the dependent variables. For neurological severity (NSS-R) scores a change score was calculated by subtracting baseline scores from post injury scores. Though some argue that analysis using change scores may be subject to regression towards the mean, according to Allison (1990) one model to account for baseline differences (i.e., RMANOVA or analysis of covariance or change score analysis) is not automatically preferable to another. The decision of which model to use should be made only after considering the empirical application of each model (Allison, 1990). Based on that guidance and after consultation with a biostatistician, it was decided that the use of a change score to account for individual variance and baseline differences between groups was appropriate for these data. The change scores were then analyzed using a univariate ANOVA. Open filed activity scores were separated into three subscales: horizontal activity, vertical activity, and center time. These subscale scores were each analyzed using repeated-measures ANOVAs. Analyses of NSS-R change scores included data for all subjects (N=143). The open field analyses included only a subset of the subjects (N= 95) because of an equipment malfunction during one cohort of subjects. Fortunately, the conditions were counterbalanced across cohorts, so remaining data are representative of all experimental conditions. All tests were two tailed using alpha= .05.

Results

The results for each dependent variable are presented in this section. Significant values and values that approach significance are presented in the text and results from all statistical analyses are presented in Appendix B.

Neurological Severity Scale – Revised (NSS-R)

An ANOVA for NSS-R change scores, using all independent variables, was conducted to determine main effects of and interactions between variables. Figure 7 presents the NSS-R change scores for the non-blasted and blasted conditions collapsing Sex, Stress, and Time. Figure 7 reveals a significant main effect for Blast, $F(1,127)=4.24$, $p= .042$, $\eta^2= .03$; blasted rats' NSS-R scores (mean change= 1.27± 0.28) increased significantly more than non-blasted rats' scores (mean change=0.47± 0.27). This analysis also revealed a significant three way interaction among Blast, Stress, and Sex, $F(1,127)=5.58$, $p= .020$, $\eta^2= .04$. Figure 8 shows this interaction. To help interpret these results, internal analyses were conducted. The first analysis examined effects of Stress alone (i.e., non-blasted rats) on NSS-R change scores for male and female rats. Figure 9 shows the results of this analysis. Stressed, Males' scores (mean change= 1.85±0.50) increased more than Stressed, Females' scores (mean change= -0.19±0.55). This Stress x Sex interaction approached significance, $F(1, 68)= 3.55$, $p= .063$, $\eta^2=.05$. The next set of analyses separated the data by Time point and Sex. These analyses are presented below.

Time one. Figure 10 presents the results of Time one analyses. The overall interaction between Blast, Stress, and Sex for NSS-R change scores was not significant. When the data were broken down by sex, the relationship between Blast and Stress became clearer. Figure 11 presents the results for Males in the Time one conditions. For

these animals the interaction of Blast and Stress approached significance, $F(1,43)=3.46$, $p=.070$, $\eta^2= .07$. Where Stress alone (mean change= 2.33 ± 0.73) and Blast alone (mean change= 1.75 ± 0.73) increased NSS-R scores compared to controls, the combination of Blast and Stress did not (mean change= 0.68 ± 0.76). Figure 12 presents the results for the Female, Time one rats. In Females, Blast significantly increased NSS-R scores, $F(1,28)=5.05$, $p=.033$, $\eta^2= .15$. Blasted rats' (mean change= 1.53± 0.59) scores increased significantly more than non-blasted rats' scores (mean change= -0.34± 0.59).

Time two. Figure 13 presents the results of Time two analyses. At this time point the interaction between Blast, Stress, and Sex for NSS-R change scores approached significance, $F(1, 56)= 3.75$, $p=.058$, $\eta2= .06$. When separated by sex the results reveal a similar pattern to the Time one conditions. Figure 14 presents the results for Males, Time two where all experimental groups increased more than controls, but these findings were not significant. Figure 15 presents the results for Females, Time two where differences were not significant. But the combination of Blast and Stress increased NSS-R scores more than other groups.

Summary. For rats measured one day after injury, exposure to blast had deleterious effects on the rats' neurobehavioral functioning. Also, psychological stress attenuated these effects in male rats and potentiated these effects in female rats.

Open Field Activity (OFA)

Separate repeated-measures analysis of variances (RMANOVAs) were conducted for each of the three OFA variables: Horizontal Activity (HA), Vertical Activity (VA), and Center Time (CT) using all four independent variables. Results of these analyses are presented below. The term "measure" is used to describe the within-subject variable.

Horizontal activity. A RMANOVA using all independent variables was conducted for horizontal activity. There were no significant overall effects for Blast or Stress alone. Figure 16 reveals that Female rats (19085.84 ± 613.10) had significantly more horizontal activity than did male rats (16497.18 ± 620.71), $F(1,79)=8.80$, $p=.004$, $\eta^2= .10$. However, Stress altered this behavior in Females. Figure 17 reveals the effects of Stress on horizontal activity. Stressed, Females (16846.04 ± 980.34) decreased horizontal activity post injury, whereas all others increased horizontal activity, $F(1,79)=7.54$, $p=.007$, $\eta^2= .09$.

Time since injury also had an effect on horizontal activity. Figure 18 presents these effects collapsed across Blast, Stress, and Sex. Rats tested 8 Days after injury had significantly more horizontal activity (18723.67 ± 613.10) than did rats tested 1 Day after injury (16859.36 ± 620.71), $F(1,79)= 4.57$, $p = .036$, $\eta^2= .06$. In fact, Figure 19 reveals that rats tested at 1 Day (16122.33 ± 701.82) decreased horizontal activity post injury, whereas rats tested at 8 Days increased horizontal activity post injury (19472.48 ± 693.21), $F(1,79)= 6.53$, $p=.013$, $\eta^2= .08$. To better understand these results, the data were separated by Time point. The results of these analyses are presented below.

Time one. A RMANOVA using only rats tested 1 Day after injury was conducted for horizontal activity. Figure 20 displays the results of this analysis. There were no significant main effects for Blast or Stress alone. However, Blast did affect horizontal activity differently for males and females. Figure 21 reveals that Blasted, Females significantly decreased horizontal activity post injury, whereas all other groups did not significantly decrease, $F(1,39)= 4.83$, $p=.034$, $\eta^2= .11$. Similarly, Stress affected horizontal activity differently for males and females. Figure 22 reveals that Stressed,

Females significantly decreased horizontal activity post injury, whereas all other groups did not significantly decrease horizontal activity, $F(1,39)=9.27$, $p=.004$, $\eta^2= .19$. Despite these significant findings, it is evident from Figure 20 that the decrease in horizontal activity for females is most pronounced in the Blast + Stress condition. Figure 23 shows the results of the internal analyses of Female, Time one, Stressed rats which reveals that the decrease from baseline to post injury, in the Blast + Stress condition, is significant compared to the Stress alone condition, $F(1,10)= 5.33$, $p= .044$, $\eta^2= .35$.

Time two. A RMANOVA using only rats tested 8 Day after injury was conducted for horizontal activity. Figure 24 displays the results of this analysis. There were no significant main effects or interactions for Blast or Stress. However, Figure 25 presents the data collapsed across Blast and Stress and reveals that at Time two, Females had greater horizontal activity (20164.94 ± 880.88) than did Males (17282.40 ± 880.88), $F(1,40)= 5.35$, $p=.026$, $\eta^2= .12$. It is interesting to note, as displayed in Figure 24, that, although not significant, horizontal activity increases in all conditions except the Blast + Stress conditions for males and females, where activity decreases.

Summary. The combination of Blast + Stress decreased horizontal activity, especially for female rats. This finding suggests that exposure to blast along with psychological stress has deleterious effects on the general health, as indexed by decreased open field activity, of female rats but not on male rats.

Center time. A RMANOVA using all independent variables was conducted for center time values. There were no significant overall effects or interactions for Blast, Stress, or Sex. Time since the injury did have an effect on center time values. Figure 26 presents these effects collapsed across Blast, Stress, and Sex. Rats tested 8 Days after

injury spent significantly more time in the center (953.45 sec ± 50.07 sec) than did rats tested 1 Day after injury (771.75 sec ± 50.69 sec), $F(1,79)=6.50$, $p= .013$, $\eta^2= .08$. In fact, as revealed in Figure 27, rats tested 8 Days after injury increased center time (to 1098.05 sec ± 64.77 sec), whereas rats tested 1 day after injury slightly decreased center time (to 727.01 sec ± 65.58 sec). This interaction was significant, $F(1,79)= 12.14$, $p=.001$, $\eta^2= .13$. Because these significant results were found, the data were separated by Time point and internal analyses were conducted. The results of these analyses are presented below.

Time one. A RMANOVA using only rats tested 1 Day after injury was conducted. Figure 28 displays the results of this analysis. There were no significant main effects for Blast or Stress. Stress did affect male and female center times differently. Figure 29 shows that Non-Stressed, Females spent significantly more time in the center (1038.32 sec ± 91.54 sec) than did Stressed, Females (659.12 sec ± 91.55 sec), whereas Stressed, Males spent more (though not significant) time in the center (764.59 sec ± 96.02 sec), than did Non-Stressed, Males (624.99 sec ± 91.55 sec). This interaction of Stress x Sex was significant, $F(1,39)=7.83$, $p=.008$, $\eta^2= .17$.

Time two. A RMANOVA using only rats tested 8 Days after injury was conducted for center time values. Figure 30 displays the results of this analysis. There were no significant main effects or interactions for Blast, Stress, or Sex. All rats increased time spent in the center from baseline (808.85 ± 60.18 sec) to post injury (1098.05 sec ± 67.08 sec), $F(1,40)=18.15$, $p<.001$, $\eta^2 = .32$. Figure 31 presents these significant results.

Summary. One day after injury, female rats showed more anxiety-like behavior, whereas male rats did not change time spent in the center. In contrast, female and male rats showed less anxiety-like behavior, as indexed by time spent in the center of the open field, one week later.

Vertical activity. A RMANOVA using all independent variables was conducted for vertical activity values. There were no significant overall effects for Blast, Stress, or Sex. Time since the injury did have an effect on vertical activity. Figure 32 presents these effects collapsed across Blast, Stress, and Sex. Rats tested 8 Days after injury had significantly more vertical activity (2103.15 ± 92.02) than did rats tested 1 Day after injury (1776.08 ± 93.16), $F(1,79)=6.23$, $p= .015$, $\eta^2= .07$. Because the effect of Time was significant, the data were separated by Time point and internal analyses were conducted. The results of these analyses are presented below.

Time one. A RMANOVA using only rats tested 1 Day after injury was conducted for vertical activity. Figure 33 displays the results of this analysis. There were no significant main effects for Blast or Stress. Stress did affect vertical activity differently for male and female rats. Figure 34 reveals that Non-Stressed, Females had significantly more vertical activity (2043.83 ± 155.74) than did Stressed, Females (1524.13 ± 155.74), while Stressed, Males had more (though not significant) vertical activity (1832.80 ± 163.34) than did Non-Stressed, Males (1703.54 ± 155.74). The interaction of Stress and Sex was significant, $F(1,40)= 4.24$, $p=.046$, $\eta^2= .10$. It is evident from Figure 33 that the decrease in vertical activity for females is most pronounced in the Blast + Stress condition (43.54% change). Figure 35 shows the results of the internal analyses of Female, Time 1, Stressed rats which reveals that the decrease from baseline to

post injury in the Blast + Stress condition (to 1065.67 ± 191.40) is significant when compared to the Females, Stress alone (i.e., non-blasted) condition (1573.00 ± 191.40), $F(1,10)= 6.99$, $p= .025$, $\eta^2 = .41$.

Time two. A RMANOVA using only rats tested 8 Days after injury was conducted for vertical activity. Figure 36 displays the results of this analysis. All rats increased vertical activity from baseline (1792.94 ± 136.39) to post injury (2413.35 ± 122.20), $F(1,40)= 16.16$, $p<.001$,$\eta^2 = .29$. Figure 37 presents these significant results. There were no significant main effects or interactions for Blast, Stress, or Sex. It is noteworthy that the Stress, No Blast, Males showed the greatest increase (80% change) in vertical activity for rats tested 8 Days after injury. The relevance of this point is addressed in the discussion section.

Summary. One day after injury, female rats exposed to Blast + Stress showed the greatest increase in depression-like behavior, as indexed by decreased vertical activity. One week later all rats showed increased vertical activity, suggesting decreased depression-like behavior.

Confirmation of Hypotheses

Specific Aim 1: To determine the effects of blast on neurobehavioral functioning and mental health-like behaviors.

Hypothesis 1a: The hypothesis that blast would deleteriously affect neurobehavioral functioning as detected by higher NSS-R scores **was confirmed**. Blasted rats had significantly higher NSS-R scores than did non-blasted rats. The results of the present experiment are consistent with the reports from clinical literature. In clinical cases patients with TBI demonstrate significantly more post-concussion symptoms, typically measured using the Neurobehavioral Symptom Inventory (Belanger, Kretzmer, Vanderploeg, & French, 2010; Belanger et al., 2011).

Hypothesis 1b. The hypothesis that blast would deleteriously affect mental health-like behaviors (including anxiety-like and depression-like behaviors) as detected by decreased center time and vertical activity for blasted animals **was not confirmed**. Blast *alone* did not significantly affect mental health-like behaviors at either time point. Blasted rats and non-blasted rats had similar open field activity for all parameters (i.e., HA, CT, VA).**Specific Aim 2:** To determine the effects of stress alone and stress in combination with blast on neurobehavioral functioning, and mental health-like behaviors.

Hypothesis 2a. The hypothesis that stress would deleteriously affect neurobehavioral functioning, as detected by higher NSS-R scores, **was not confirmed**. Stress *alone* did not affect neurobehavioral functioning. Stressed and non-stressed rats performed similarly on the NSS-R.

Hypothesis 2b: The hypothesis that stress would deleteriously affect mental health-like behaviors (including anxiety-like and depression-like behaviors), as detected by

decreased center time and vertical activity for stressed animals, **was not supported**. Stress *alone* did not affect mental health-like behaviors. Stressed and non-stressed rats had similar locomotor activity.

Hypothesis 2c: The hypothesis that stress would either exacerbate or attenuate the effects of blast on neurobehavioral functioning and mental-health like behaviors **was not confirmed**. Stress and blast alone (i.e., without considering sex) had no effects on neurobehavioral functioning or mental health-like behaviors. Stress did affect males and females differently and the combination of blast and psychological stress had different effects for male and female rats. These findings are discussed under Specific Aim 3 and its hypotheses.

Specific Aim 3: To examine sex as an individual difference variable that may change responses to blast and stress and the effects the two variables have on neurobehavioral functioning and mental-health like behaviors.

Hypothesis 3a: The hypothesis that blast would affect males and females differently on measures of neurobehavioral functioning and mental health-like behaviors **was partially confirmed** by trends found in the data**.** Blasted males and females showed similar increases in neurobehavioral severity, but differed on mental health-like behaviors. Blast alone increased NSS-R scores for both male and female rats (Figure 8). Blasted males showed no change on center time or horizontal activity and only a slight increase on vertical activity. In contrast, blasted females had deceased center time, vertical activity, and horizontal activity.

Hypothesis 3b: The hypothesis that stress would affect males and females differently on measures of neurobehavioral functioning and mental-health like behaviors

was confirmed. Stress alone increased NSS-R scores for males and decreased scores for females. For males, stress alone slightly increased horizontal activity, but did not change center time or vertical activity. In contrast, for females, stress alone decreased horizontal activity and center time and did not change vertical activity.

Hypothesis 3c. The hypothesis that blast and stress in combination would affect males and females differently on measures of neurobehavioral functioning and mental-health like behaviors **was confirmed**. For neurobehavioral function the blasted, stressed females had worse NSS-R scores than females in all other conditions. In contrast, blasted, stressed male NSS-R scores were similar to non-blasted, non-stressed males.

Specific Aim 4: To determine if time is important for the measurement of neurobehavioral functioning and mental-health like behaviors following blast.

Hypothesis 4. The hypothesis that measurements of neurobehavioral functioning and mental-health like behaviors taken 1 day following blast would be different from measurements taken 8 days after blast exposure was **partially confirmed**. Measurements of mental health-like behaviors were different for 1 day and 8 days post injury, but neurobehavioral functioning was similar for both time points. Rats had less activity 1 day post injury than 8 days post injury.

Internal analyses revealed sex differences within the time points and differences between the two time points because of stress. For time one, the female rats' depressive-like, and anxiety-like behaviors significantly increased from baseline measures, whereas male mental health-like behaviors did not change significantly from baseline. Additionally, female stressed rats in the time point one condition showed more depressive-like and anxiety-like behaviors. Time point two rats showed different patterns

in mental health-like behavior. Both males' and females' anxiety-like behaviors decreased from baseline. Males also decreased depression-like behavior from baseline. Instead of interpreting this change in activity for males as decreased depression, it may be explained as increased escape behavior. This alternate interpretation would be consistent with the male stress response of "fight-or-flight." Males increased escape behavior over time in response to stress. Females showed no significant change in depression-like behavior. Stress did not seem to have any effects at time point two.

Discussion

This experiment used a rat model to determine how stress alters behavioral effects of blast-induced injury in male and female rats at two time points post injury. Four independent variables were manipulated in this experiment: injury (no blast, blast); stress (no stress, stress); sex (male, female); and time (1day and 8 days post injury). The blast over pressure (BOP) paradigm was used to induce blast injury. The Warrior Stress Paradigm, a combination of predator and environmental stressors, was used to stress the rats. The dependent variables were a measure of neurobehavioral functioning (NSS-R) and indices of mental health-like behaviors (including anxiety-like and depression-like) measured using open-field locomotor activity. This work included between-subjects and within-subject comparisons of behavior measured before and after injury. The following paragraphs provide commentary on the specific aims, discuss the findings in general, the limitations of this work, and the future directions for these research questions.

Commentary on Specific Aims

This research project had four specific aims with one to three hypotheses associated with each specific aim. Confirmation of these hypotheses was presented above. The following section provides discussion of the findings associated with each specific aim.

Specific Aim 1. With regard to Specific Aim 1, the effects of blast on behavior, the present work established that the BOP model can be used to induce measurable neurobehavioral changes. The present experiment used a new measure of neurobehavioral functioning, the NSS-R, to reliably detect the effects of blast on behavior. This series of behavioral measures provides a reliable and relatively simple

way to assess the effects of injury on sensory and motor reflexes in rodents. This test is the only one of its kind used to detect behavioral effects following blast injury in rodents. Readnower and colleagues (2010) are the only other group to use a similar test following exposure to blast. However, they tested only three reflexes (righting response, eye reflex, and paw flex) in blasted and control rats. The present experiment examined ten neurobehavioral reflexes, 24 hours and eight days after injury, in male and female rats and found substantial effects. It is noteworthy that blast did not alter performance on the balance task within the NSS-R battery; therefore, it is unlikely that other behavioral effects of blast could be attributed to effects of blast on the vestibular system.

Similar tests of "neurological severity" (e.g., NSS; "neurological" is the term that has been used in the literature even though "neurobehavioral" is probably more accurate) have been used to detect effects of injury in other animal models of neurological injury (e.g., Hamm, 2001; Mahmood et al., 2001; Shohami et al., 1995). The NSS-R was designed to increase reliability and sensitivity to detect behavioral effects of injury. The results of the present experiment using the NSS-R are consistent with the findings of others. Cole and colleagues (2011) used the NSS-R to show that "sham" injuries, for the controlled cortical impact model, do affect neurobehavioral functioning and their use should be carefully considered when designing an experiment. They found that "sham" injuries resulted in greater NSS-R scores than naives. The present experiment used the NSS-R to detect injury following a blast. The BOP paradigm was used because it "…

realistically resembles the conditions under which troops are exposed to blast on the battlefield, and also provides survivable blast conditions under which brain injury can be generated and studied" (Long et al., 2009, p 828). The use of this paradigm (i.e.,

rather than other models) in the present experiment may be a better way to investigate human bTBI in animals. This experiment also improves on others like it that measure neurobehavioral severity following injury, because it investigates the addition of psychological stress and the effects in male and female rats. This experiment also provides valuable information about behaviors that are used as indices of mental health following injury. Warriors exposed to blast are also exposed to psychological stress before, during, and after injury. In humans it is impossible to separate these variables. However, in a true experiment, like the present work, blast and stress are investigated separately and in combination. The ability to separate these variables provides valuable information about the relationship between blast, stress, and mental health problems. The results of the present work are consistent with other reports from one group that conducts similar investigations of blast injury, using male, Sprague-Dawley rats, of a similar age. They found no significant difference between blasted rats and non-blasted rats on time spent in the open arms of the elevated plus maze in two separate experiments (Kamnaksh et al., 2011; Kovesdi et al., 2011). This group used only male rats and did not add psychological stress in these experiments. The present experiment included psychological stress in combination with blast in male and female rats to determine if psychological stress altered the affects of blast and explained its relationship with mental health problems. The interactions of these variables are discussed under Specific Aim 3.

Specific Aim 2. There were no significant findings associated with Specific Aim 2 that investigated the effects of stress alone on behavior. The combination of blast and stress did affect male and female rats differently on measures of neurobehavioral

functioning, center time, and vertical activity; these results are discussed under Specific Aim 3.

Specific Aim 3. With regard to Specific Aim 3, this experiment found that males and females had different responses to blast, stress, and the combination of the variables. The deleterious effects of blasts on neurobehavioral responses (discussed under Specific Aim 1) were attenuated by psychological stress in males and potentiated by psychological stress in females. Additionally, mental health-related behavior was affected differently by the combination of blast and psychological stress for males and females. For males, the combination of blast and stress slightly increased horizontal activity, center time and vertical activity. In contrast, blasted, stressed, females had significantly decreased center time and vertical activity. The results for males are inconsistent with the reports from Kwon and colleagues (2011). This group used male, Sprague-Dawley rats of a similar age and used a similar stressor in addition to blast injury. They found that injured, stressed rats had decreased center time on the elevated plus maze (Kwon et al., 2011). As previously discussed, the elevated plus maze may be a more sensitive measure of anxiety that might explain the inconsistent findings. The Kwon group did not examine effects in female rats, so a full comparison with the present experiment is not possible.

The sex differences for the effect that blast and stress had on neurobehavioral functioning and mental health-like behaviors found in the current experiment demonstrate the need for investigating these variables (i.e., blast and stress) in both male and female rats. A recent report from the clinical literature found that female Veterans with a history of deployment related-TBI were more likely than males to have a diagnosis of depression, anxiety disorder, PTSD with comorbid depression, and reported more severe

neurobehavioral symptoms (Iverson et al., 2011). This report may indicate that there are sex difference in neurological and psychological reactions to TBI; alternatively these differences may be accounted for by factors other than gender that were not considered (e.g., history of trauma). Based on the sex differences found in the present study along with the possibility of sex differences in Iverson et al. (2011), it is important to include male and female subjects in future investigations of TBI.

Specific Aim 4. With regard to Specific Aim 4, the effects of time on behavioral responses to blast and stress, this experiment found that the deleterious effects on mental health-like behavior, present one day after injury in females, disappeared for all animals one week after injury. These results stand in contrast to the findings of Kwon and colleagues (2011) who reported that it may take months for anxiety-like behaviors to return to normal following blast exposure. These investigators used a more intense blast exposure (about 120% of blast used in the present experiment); longer duration of stress exposure (14 days vs. 7 days in the present experiment); and a different method for indexing anxiety-like behavior (elevated plus maze vs. center time; Kwon et al., 2011) which may explain the difference in findings. There also may be other factors (e.g., social support; return to duty; also prior exposure to blast; exposure to traumatic stress) that contribute to the development of symptoms after blast exposure (Fourtassi et al., in press; Belanger et al., 2011) that were not manipulated in the present experiment.

General Discussion

This experiment set out to determine if blast affected neurobehavioral functioning using the NSS-R. Blast alone did deleteriously affect neurobehavioral functioning. This experiment also was designed to determine if blast had different effects on behavior of

male and female rats. On measures of neurobehavioral functioning, males and females were similarly affected by blast alone. However, the combination of blast and stress revealed a different pattern of results for males and females. For males, the exposure to a threat in addition to the blast had an attenuating effect. This finding is consistent with the interpretation that the "fight or flight" response to a threat, serves as a survival function to protect the individual rat (Cannon, 1935). In females, the addition of a threat to the blast potentiated its effect. These findings demonstrate that the animal model paradigms of blast and stress used in this experiment do alter neurobehavioral behaviors and should be used in future investigations. These findings also show that the NSS-R can be used to measure neurobehavioral functioning following brain injury in rodents. Additionally, these findings illustrate the necessity for using both male and female rats in any future experiments.

Another goal of this experiment was to determine if psychological stress altered the behavioral effects of blast, a change that might explain the development of mental health problems following blast injury. Interestingly, the blast alone had no effects on mental health-like behaviors. It was only in combination with psychological stress that differences became apparent, and these differences were only detected in female rats. For females, the addition of psychological stress to the blast resulted in more depression-like and anxiety-like behaviors. This pattern of results was not found in the male rats. Male rats demonstrated little change in mental health-like behaviors in response to any experimental manipulation. These null findings in male rats have implications for other similar investigations of blast injury that use only male rats. Future experiments should include both males and female rats. The change in behaviors as a result of the

combination of blast and stress in female rats illustrate the need for including measures of psychological stress in future experiments. Additionally, it would be valuable to include a variety of stressors in future research.

Limitations

Independent variables. There are a few limitations to the current experiment's independent variables. With regard to blast, only one level of blast was used and the rats were blasted only one time. To better model the human condition, more information is need about the level and number of blasts to which Warriors are exposed. Additionally, there were only two levels of the injury variable (i.e., no blast, blast). Based on the work of Kamnaksh and colleagues (2011), the stress of travel and anesthesia, associated with exposure to blast, may contribute to changes in behavior. Therefore, another travel, anesthesia, control group that is not blasted may be important to include in future experiments using this blast paradigm. It also is worth considering whether any behavioral or psychological effects of blast exposure are related to the size and structure of the brain. If so, then caution should be exercised in attempts to extrapolate findings using rat models to humans.

With regard to stress, this experiment only evaluated one type of stress paradigm. Other stress paradigms may yield different behavioral responses, and may be relevant to Warriors (e.g., sleep disruption; Perry, 2009).

With regard to the timeline used by the present experiment, it may not have been long enough. Two time points were included (one and eight days post injury) to get some idea about whether time was a factor in the behavioral effects of blast. Ideally, this study

should have included many time points ranging from immediately after blast to several months.

Dependent Variables. There also were limitations associated with the dependent variables of this master's project. The experiment described here is part of a large study that investigates behavioral and biological effects of blast and stress in male and female rats at two time points. Presenting the experiment in its current form (i.e., with only two behavioral outcomes) may limit the full understanding of the effects of blast and stress for male and female rats on neurobehavioral functioning and mental health-like behaviors. Other behaviors that are included in the large study and related experiments (e.g., acoustic startle response and hot plate) would provide information about PTSD-like and pain responses, respectively. These measures are relevant to the human condition where PTSD and pain (especially migraine) are strongly associated with blast and stress. In addition, the larger experiment includes biological variables from blood (stress hormones; corticosterone, ACTH, prolactin) and brain samples (protein and neurotransmitter levels from specific regions) that could be combined with the behavioral measures to provide a complete picture of the animals response to blast and stress. Other potential changes to the dependent variables are discussed in the Future Directions section below.

Future Directions

The present work has established that the BOP rat model can be used to induce measurable brain injury. This work also has demonstrated the utility of the NSS-R in detecting damage caused by blast injury. Additionally, the Warrior Stress Paradigm successfully altered open field behavior in female rats. These methods can be used in

future investigations that model brain injury to gain more information for the prevention, detection, and treatment of brain injury. This work may be improved by changes or additions to the independent variables and extended by changes or additions to the dependent variables.

Independent variables. The next step for modeling blast should include exposure to multiple mild blast injuries. Reports from the field indicate that Warriors are commonly exposed to multiple blasts (especially from IEDs; Barry, 2011; Watson, 2011) and that multiple blast exposures may be particularly harmful (Kamnaksh, et al., 2011; Rosenfeld & Ford, 2010; Ruff, Ruff, & Wang, 2011). Future experiments should combine multiple blast exposures with the Warrior Stress Paradigm and use the NSS-R and open field to detect effects on neurobehavioral functioning and other behaviors. The use of a travel, anesthesia control group also will help to control for any confounds with the stress of the blast exposure process.

The present work also could be extended to include other models of stress, such as sleep disruption/deprivation in combination with TBI. Deployed Warriors are subject to sleep disruption and deprivation that affects their performance and behavior. Perry (2010) used an animal model to study the deleterious effects of sleep disruption on behavior. This stressor could be added to the blast injury to provide a more complete model of the Warrior's experience of stress that occurs in addition to the brain injury.

Another independent variable that could be improved is the time variable. Future experiments should extend the amount of time between injury and post injury measures. Patients who experience lasting effects of blast are reporting symptoms much longer than a year (U.S. Department of Defense and Department of Veterans Affairs, Traumatic

Brain Injury Task Force, 2008). Therefore, the experiment that attempts to measure these symptoms should be extended beyond a "one year" time point.

Dependent variables. This work could be extended by adding other behavioral assays that measure similar psychological constructs and other psychological constructs. Anxiety-related behaviors were measured in this experiment using the amount of time spent in the center of an open field. This behavior also can be measured using an elevated plus maze (e.g., Elliot et al., 2004). Additional assays of anxiety-related behavior using the elevated plus maze may provide more information about the effects of blast and stress and their combination on anxiety-related behaviors. Depression-related behavior was measured in this experiment using the amount of escape behaviors in an open field. Depression-related behavior also can be indexed using a learned helplessness paradigm or the forced swim test (e.g., Carlezon et al., 2002; Detke, Rickels, & Lucki, 1995; Petit-Demouliere, Chenu, & Bourin, 2005; Pliakas, 2001; Porsolt, Le Pichon, & Jalfre, 1977). Adding this measure would provide more information about the effects of blast, stress, and their combination on depression-related behaviors.

To gain a more complete picture of the effects of blast, stress, and their combination on behavior, additional measures should be included that index other psychological constructs. Cognitive domains of interest include memory, learning, and attention. These constructs can be measured using passive avoidance tasks (Dubrovina, 2006) and acoustic startle response measures (Swerdlow, Caine, Braff, & Geyer, 1992). Other behaviors of interest include impulsivity and drug abuse.

Summary

The present work attempted to model the effects of blast, stress, and their combination in male and female rats at two time points. In its current form the experiment provides information about one popular rodent model of blast injury and a new model of psychological stress. This experiment demonstrates that these models can be used in future experiments of blast-induced injury to attempt to improve prevention, detection, and treatment for patients with bTBI.

Additional study is needed to determine the exact contribution of blast and stress to the development of mental health problems following injury. The results of this experiment suggest that psychological stress can attenuate the effects of blast in male rats while potentiating these effects in female rats. If this phenomenon is true in human patients, then traumatic response and medical treatment practices in theatre and at higher echelons of care should be different for males and females. Male TBI patients may require an increase in psychological arousal following injury, whereas females may need psychological arousal to be decreased. More investigation into the biological mechanisms involved (e.g., stress hormones) in these differential effects may provide additional insight into exactly how the patients should be treated. The present experiment clearly demonstrates sex differences in response to bTBI and stress.

Conclusions

Psychological stress attenuated behavioral effects of blast-induced injury in male rats, but potentiated behavioral effects of blast-induced injury in female rats. The BOP model induced brain injury in rats as measured by increased NSS-R scores in blasted animals. Additionally, time played a role in mental health-like behaviors with greater amounts of anxiety-like and depression-like behaviors one versus eight days post injury.

APPENDIX A- FIGURES

FIGURE 1

Blast Over Pressure Shock Tube

Photo courtesy of M. Shaughness, WRAIR/NMRC

FIGURE 2

Warrior Stress Paradigm Equipment

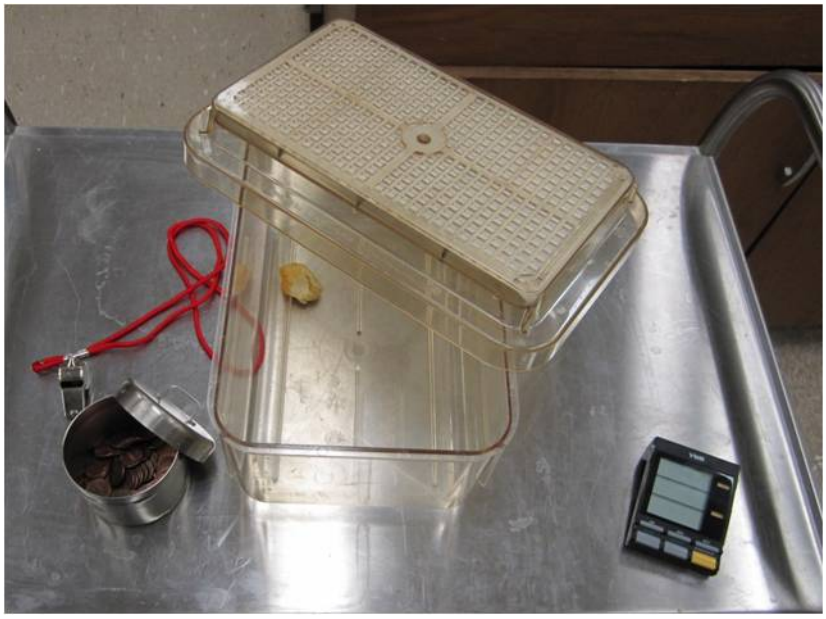


Photo by A. Yarnell

FIGURE 3

Warrior Stress Paradigm Timeline

DAY 1	PREDATOR STRESS 20 MIN
DAY 2	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN WHISTLE @ 12, 15, AND 19 MIN
DAY 3	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN COIN SHAKE @ 11, 14, AND 17 MIN
DAY 4	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN FLASHING LIGHTS @ 13, 16, 18, AND 19 MIN
DAY 5	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN CAGE SHAKE @ 12, 15, AND 18 MIN
DAY 6	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN FLASHING LIGHTS @ 12, 16, AND 19 MIN
DAY 7	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN WHISTLE @ 11, 13, 16, AND 18 MIN

FIGURE 4a

Neurological Severity Score Sheet

NSS-R

DATE Time _____ Rater _____

*Please circle one of the values per subject

SUBJECT # _____

1. General Balance

0	1	2
balance & walk	balance no walk	no balance/ fall

2. Landing Test

0	1	2
normal landing	partial compromised	no reflex/ falls flat

3. Tail Raise Test

0	1	2
normal reflex	partial/ weak reflex	no reflex/ limp

4. Drag Test

0	1	2
walking motion w/forepaws	partial/ unilateral response	no response

5. Righting Reflex

0	1	2
instant response	delayed/ takes effort	no response

6. Ear Reflex

0	1	2
full response	partial response	no response

7. Eye Reflex

0	1	2
eyeblink	partial response	no response

8. Sound Reflex

0	1	2
flinch then walk	slow mvt &/or no freeze	no response

9. Tail Reflex

0	1	2
turn/bite	turn/no bite	no response

10. Paw Flexion Reflex

0	1	2
turn/bite	turn/no bite	no response

FIGURE 4b

Neurological Severity Score

Photo by A. Yarnell

FIGURE 5

Open Field Activity Chamber

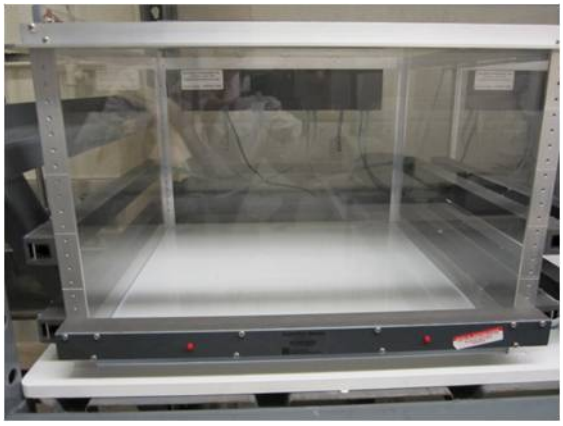


Photo by A. Yarnell

FIGURE 6

Experimental Timeline

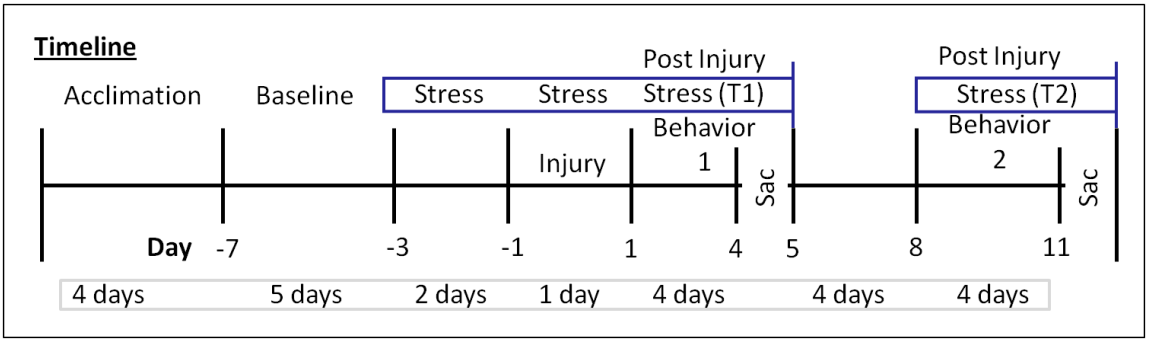


FIGURE 7

Effects of Blast on NSS-R change scores

$F(1,127)=4.24$, $p= .042$, $\eta^2= .032$

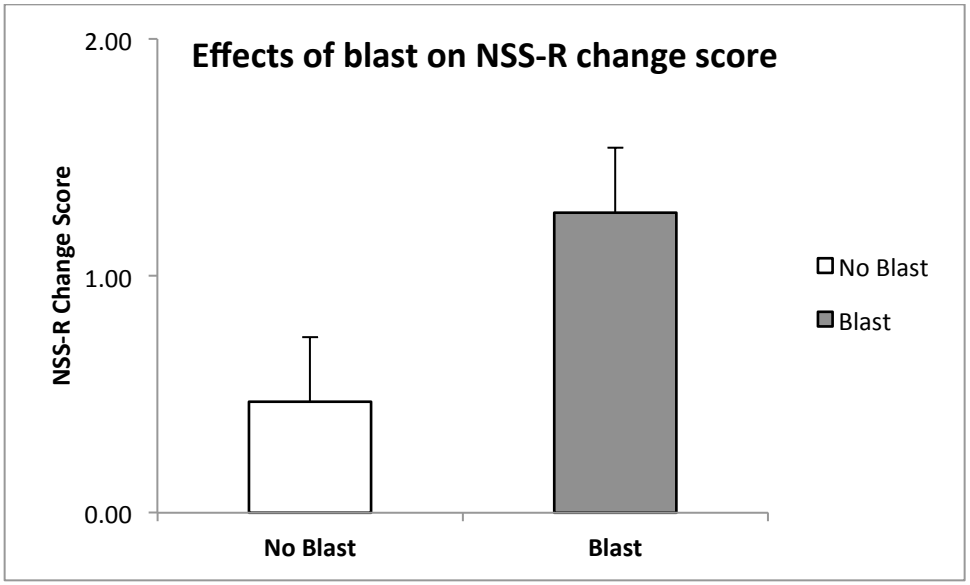


FIGURE 8

Effects of Blast and Stress on NSS-R Change Scores

$F(1,127)=5.58$, $p= .02$, $\eta^2= .04$

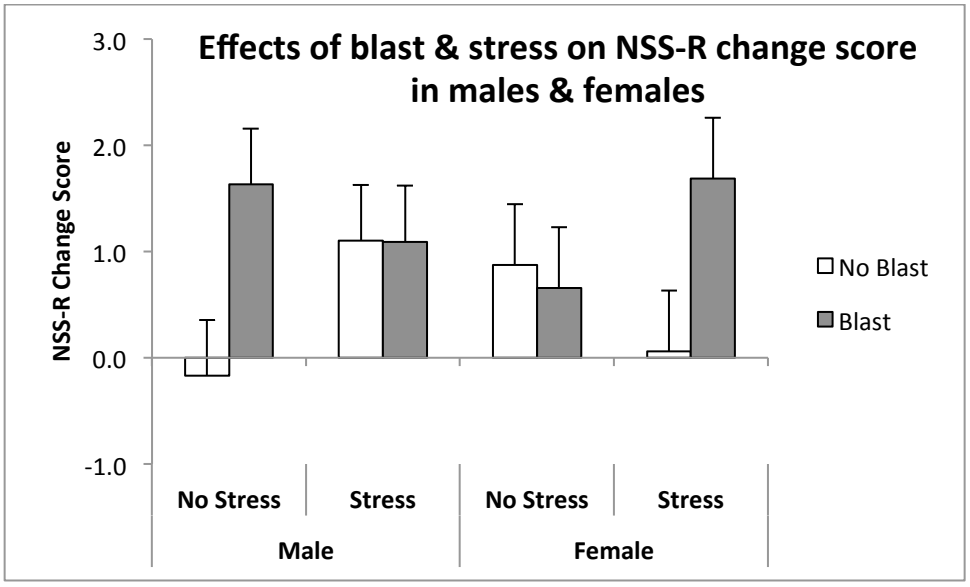


FIGURE 9

Effects of Stress alone on NSS-R change scores for male and female rats

$F(1, 68)= 3.55$, $p= .063$, $\eta^2=.050$

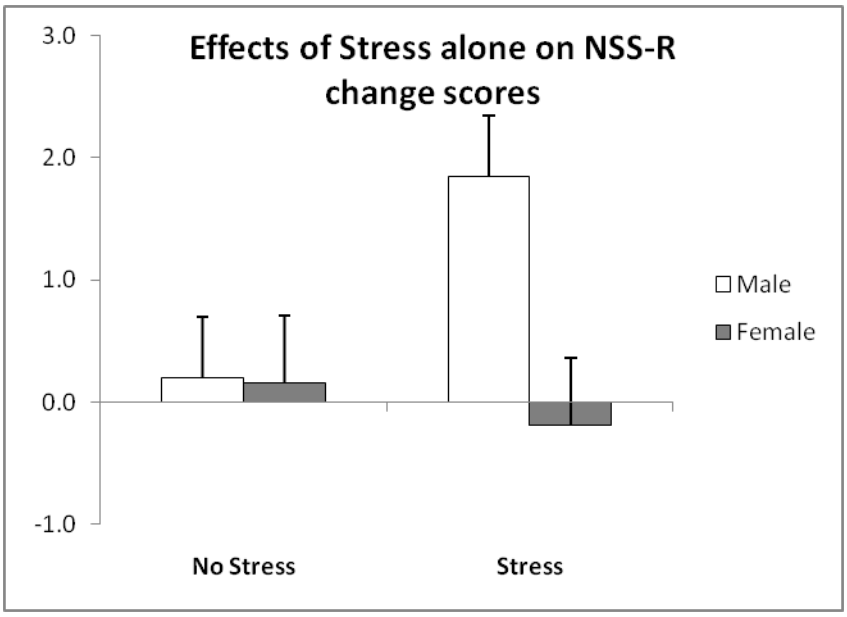


FIGURE 10

Time 1 NSS-R change scores

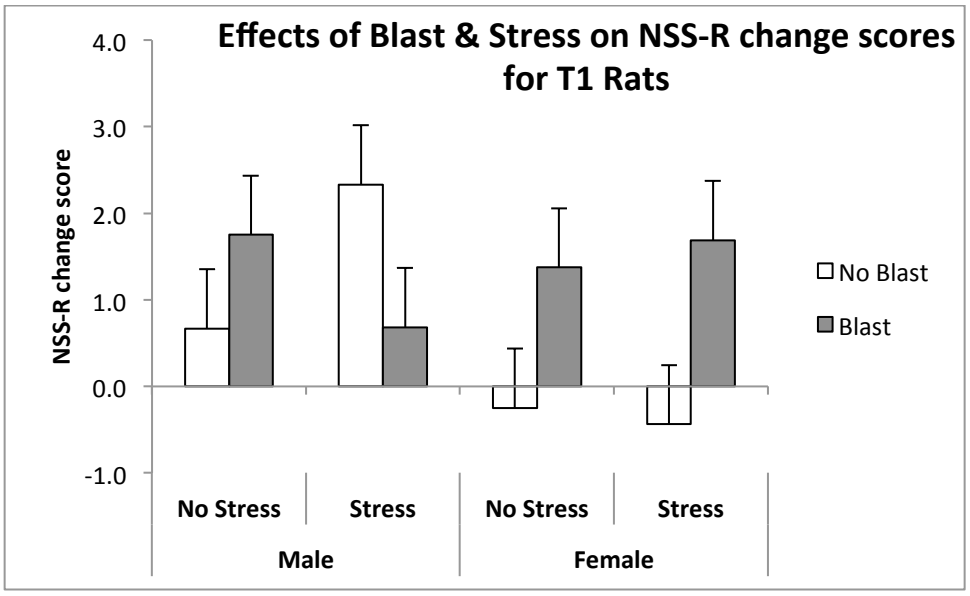


FIGURE 11

Effects of Blast and Stress on NSS-R Change Scores for Males, Time 1

$F(1,43)=3.46$, $p=.070$, $\eta^2= .070$

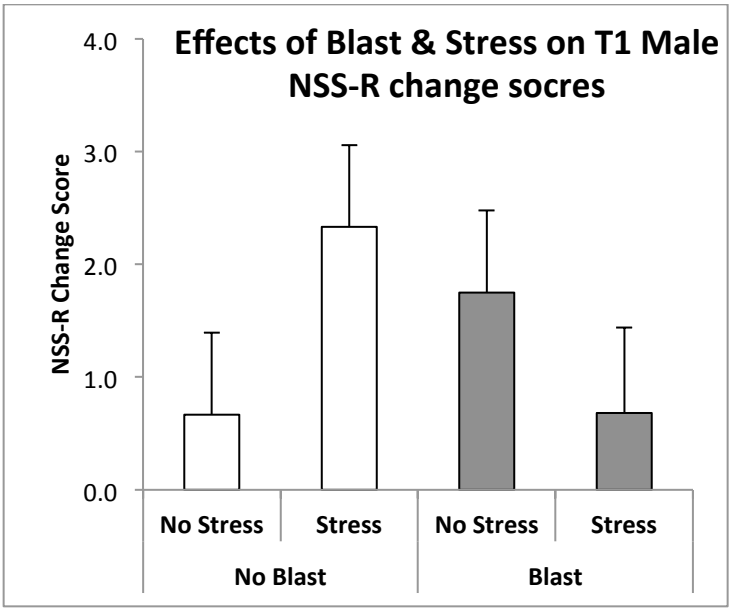


FIGURE 12

Effects of Blast and Stress on NSS-R Change Scores for Females, Time 1

$F(1,28)=5.05$, $p=.033$, $\eta^2= .153$

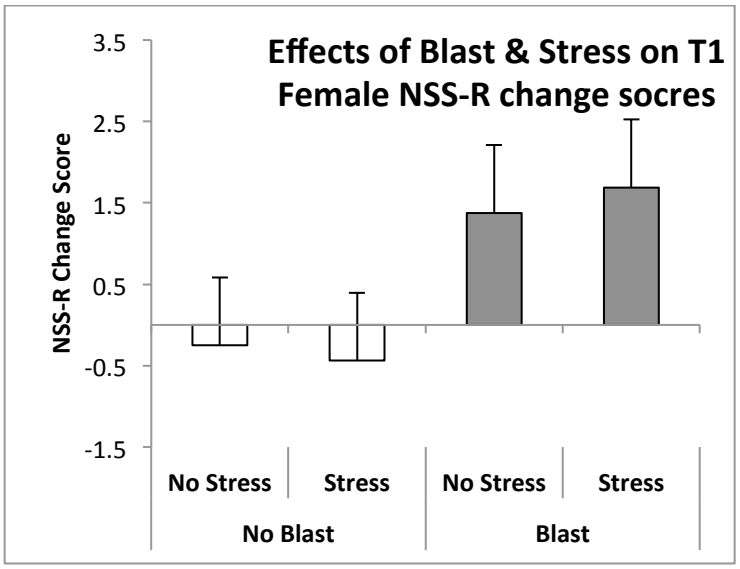


FIGURE 13

Effects of Blast and Stress on NSS-R change scores for Time 2 rats

$F(1, 56)= 3.75, p=.058, \eta^2= .063$

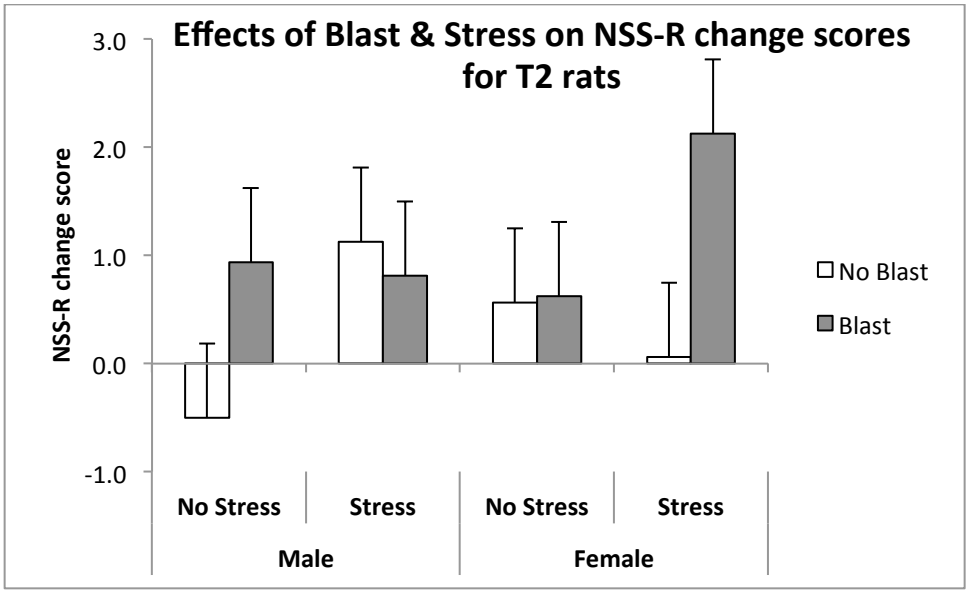


FIGURE 14

Effects of Blast and Stress on NSS-R change scores for Males, Time 2

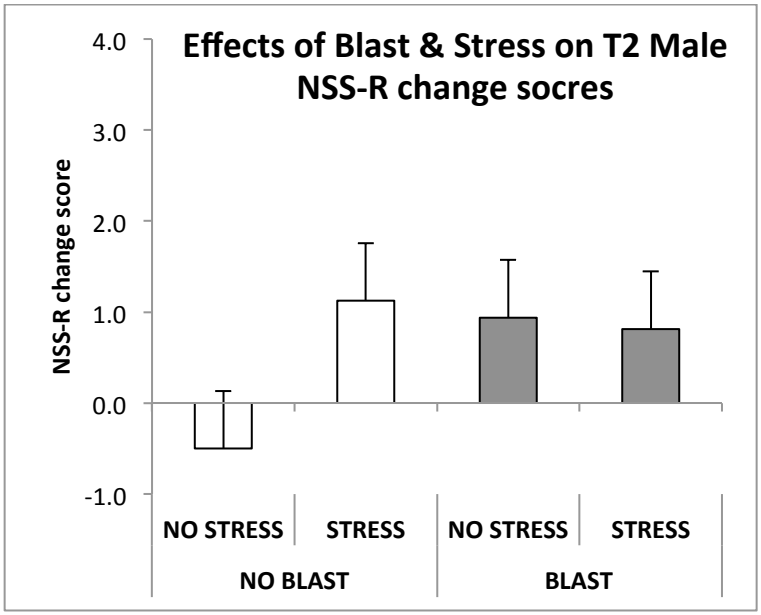


FIGURE 15

Effects of Blast and Stress on NSS-R change scores for Females, Time 2

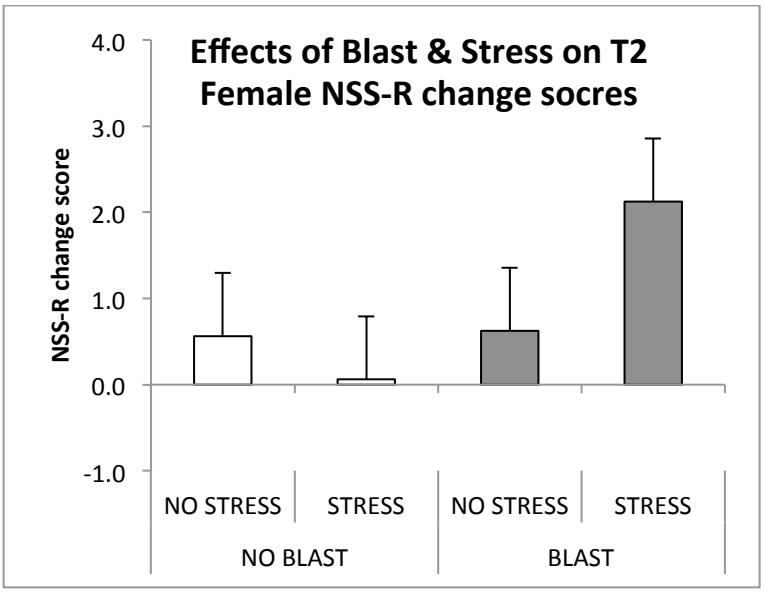


FIGURE 16

Horizontal activity for Male and Female rats collapsed across Blast, Stress, & Time

$F(1,79)=8.80$, $p=.004$, $\eta^2= .100$

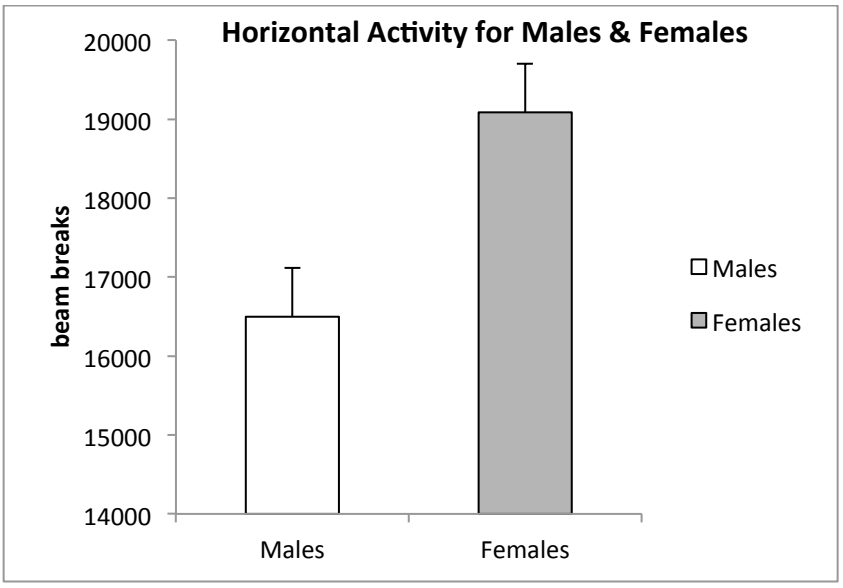


FIGURE 17

Effects of Stress on Horizontal activity collapsed across Blast and Time

$F(1,79)=7.54$, $p=.007$, $\eta^2= .087$

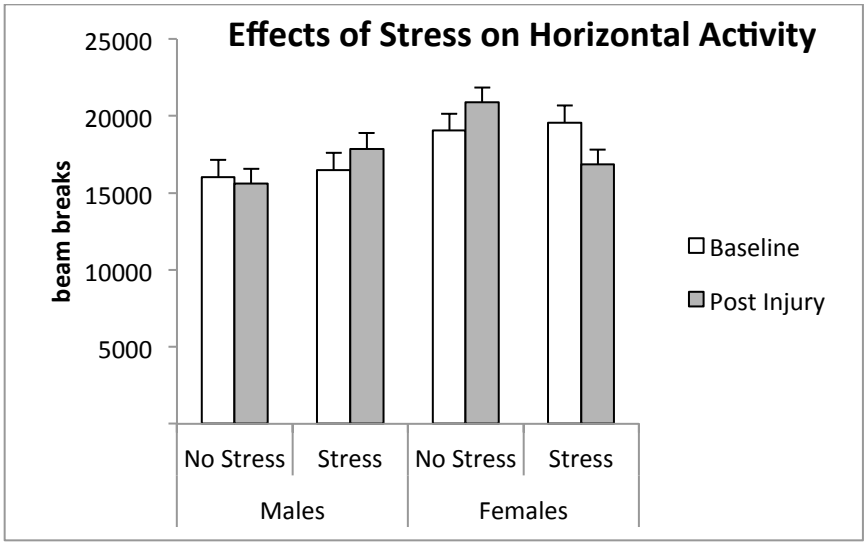


FIGURE 18

Effects of Time on Horizontal Activity collapsed across Blast, Stress, and Sex

$F(1,79)= 4.57$, $p = .036$, $\eta^2= .055$

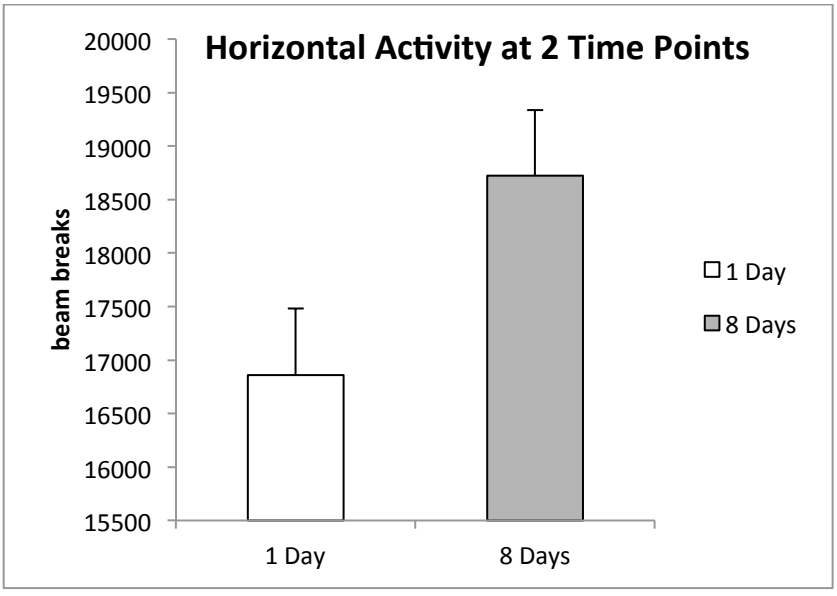


FIGURE 19

Effects of Time on Horizontal Activity collapsed across Blast, Stress, and Sex

$F(1,79)= 6.53$, $p=.013$, $\eta^2= .076$

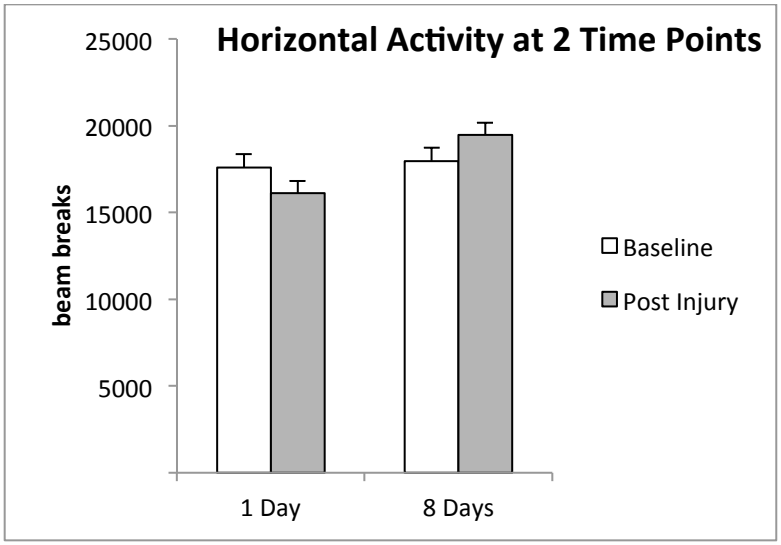


FIGURE 20

Effects of Blast and Stress on Horizontal Activity one day after injury

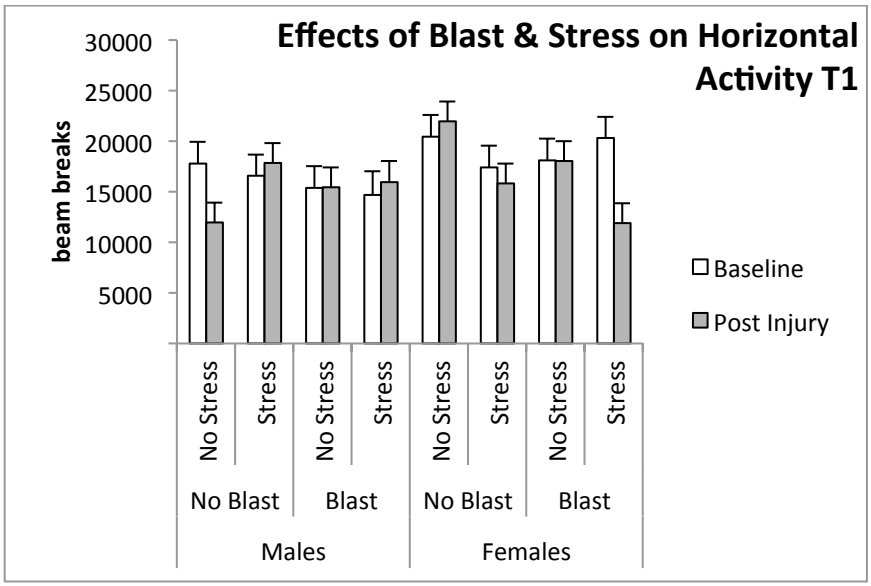


FIGURE 21

Effects of Blast on Horizontal Activity one day after injury

$F(1,39)= 4.83$, $p=.034$, $\eta^2= .110$

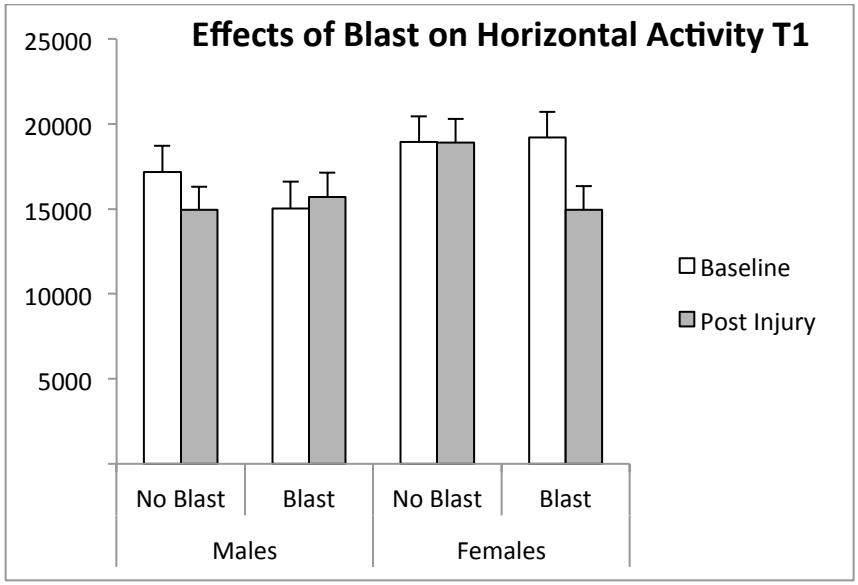


FIGURE 22

Effects of Stress on Horizontal Activity one day after injury

$F(1,39)=9.27$, $p=.004$, $\eta^2= .192$

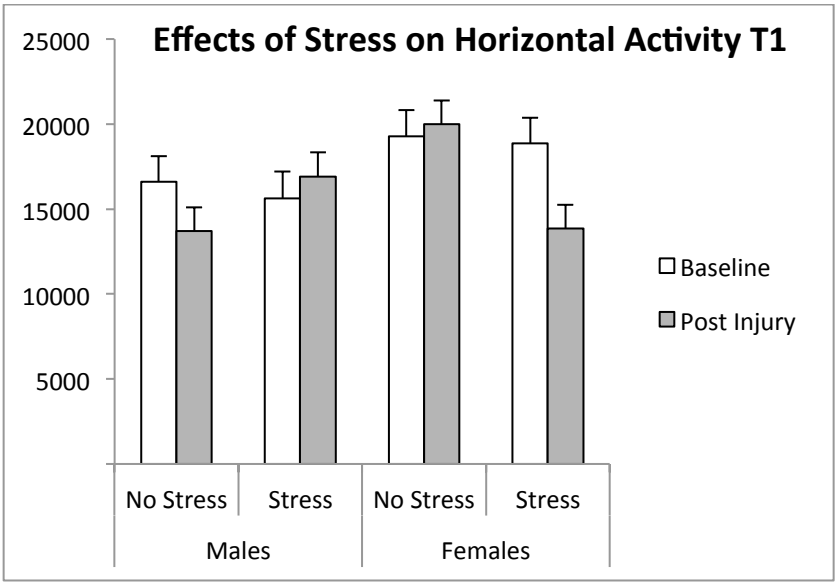


FIGURE 23

Effects of Blast on Horizontal Activity for Stressed, Females one day after injury

$F(1,10)= 5.33$, $p= .044$, $\eta^2= .348$

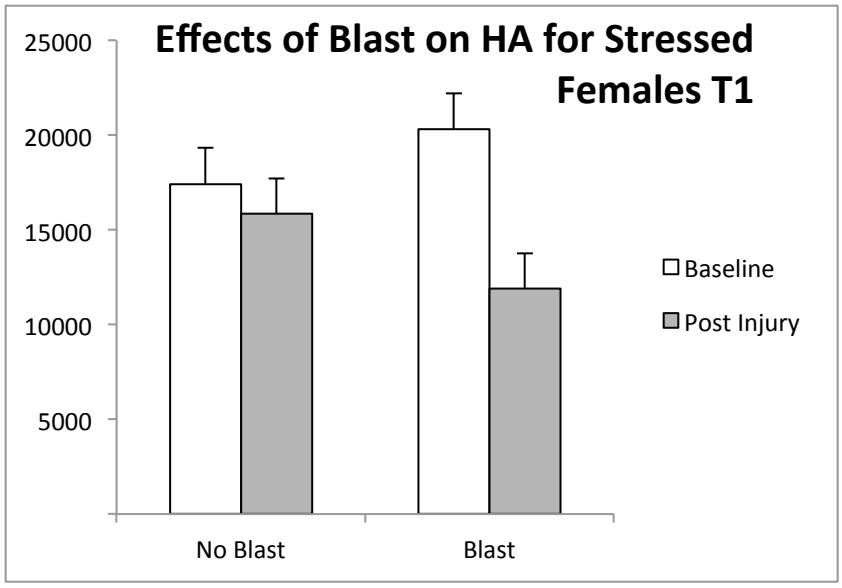


FIGURE 24

Effects of Blast and Stress on Horizontal Activity eight days after injury

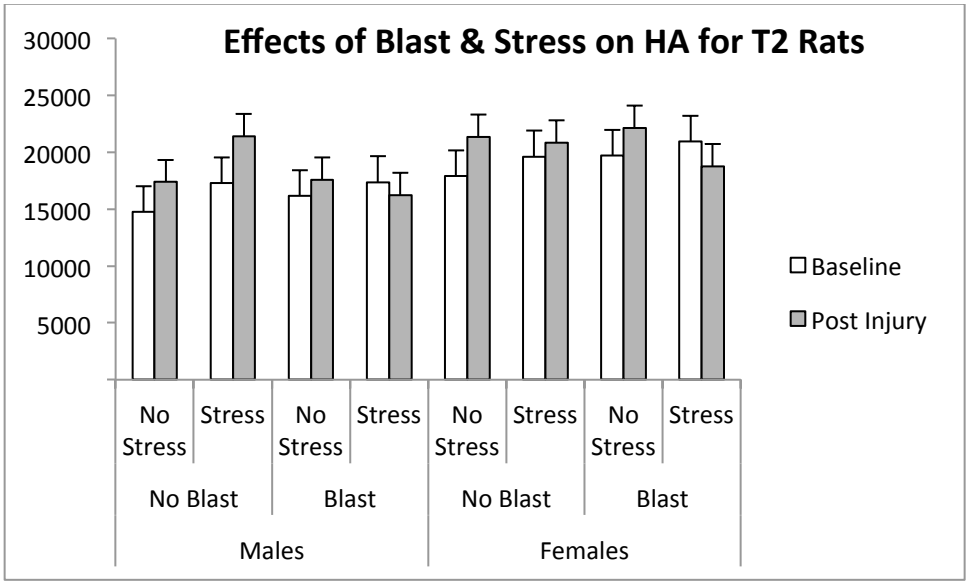


FIGURE 25

Effects of Time on Horizontal Activity eight days after injury

$F(1,40)= 5.35$, $p=.026$, $\eta^2= .118$

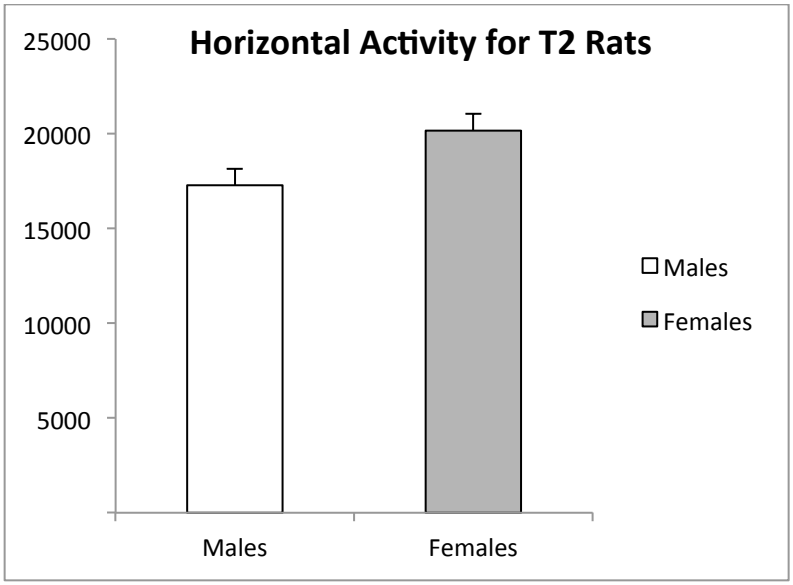


FIGURE 26

Effects of Time on Center Time collapsed across Blast, Stress, and Sex

$F(1,79)=6.50$, $p= .013$, $\eta^2= .076$

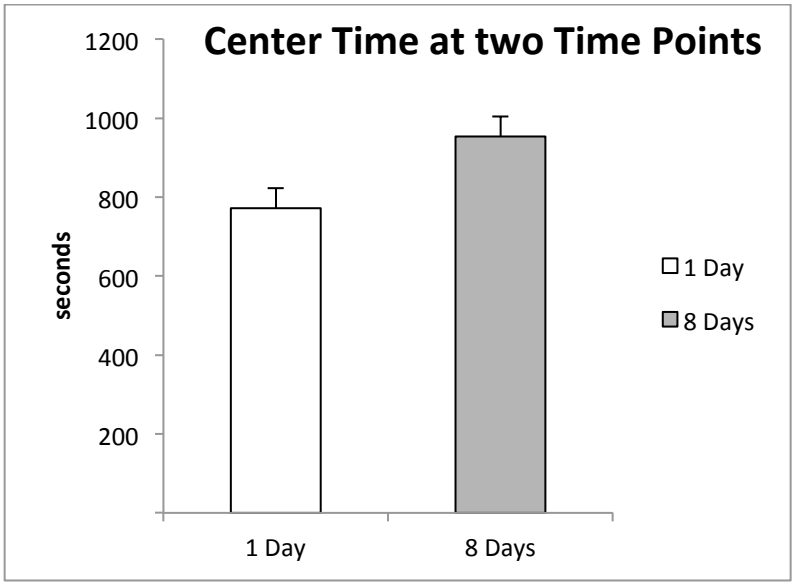


FIGURE 27

Effects of Time on Center Time collapsed across Blast, Stress, and Sex

$F(1,79)= 12.14$, $p=.001$, $\eta^2= .133$

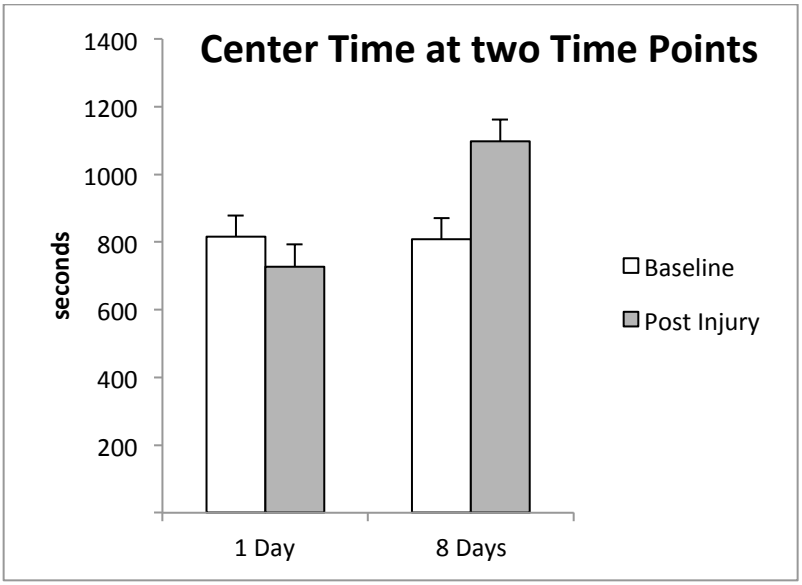


FIGURE 28

Effects of Blast and Stress on Center Time one day after injury

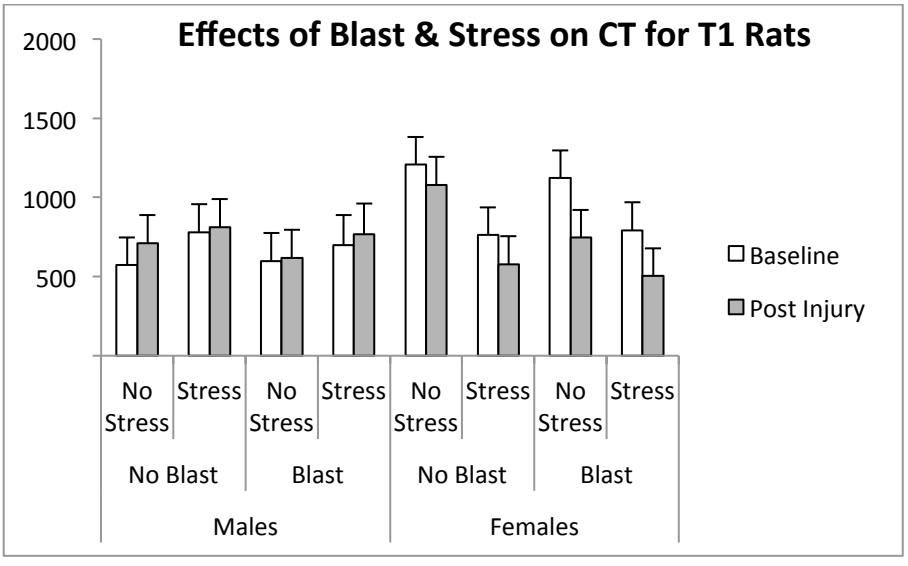


FIGURE 29

Effects of Stress on Center Time one day after injury collapse across Blast

$F(1,39)=7.83$, $p=.008$, $\eta^2= .167$

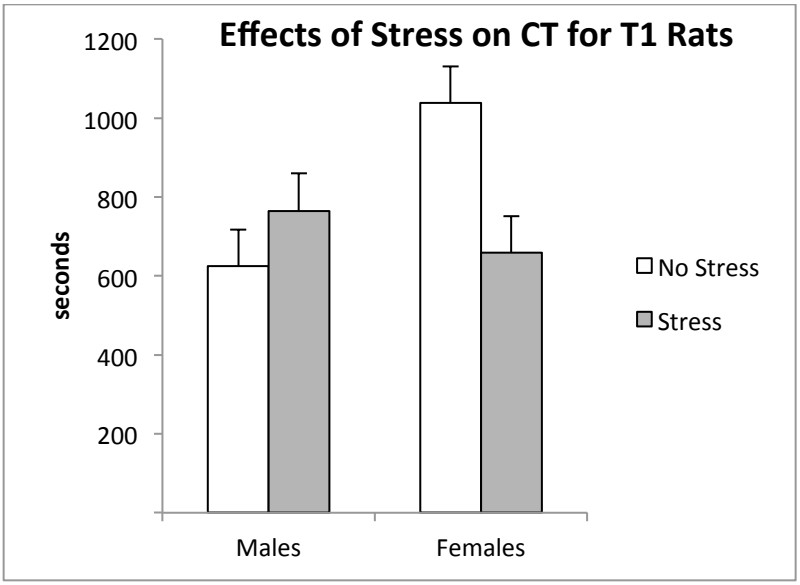


FIGURE 30

Effects of Blast and Stress on Center Time eight days after injury

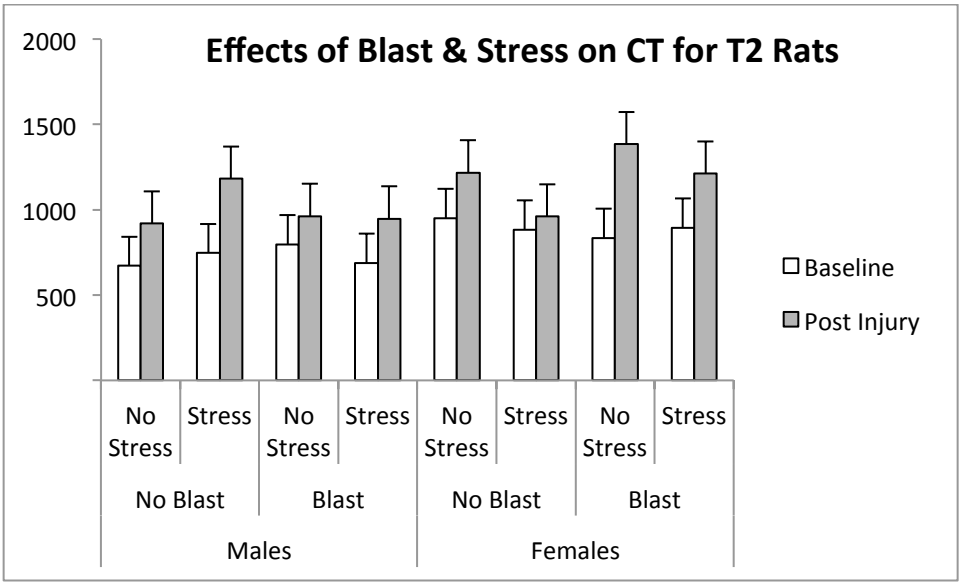


FIGURE 31

Change in Center Time from baseline to eight days after injury

$F(1,40)=18.15$, $p<.001$, $\eta^2 = .312$

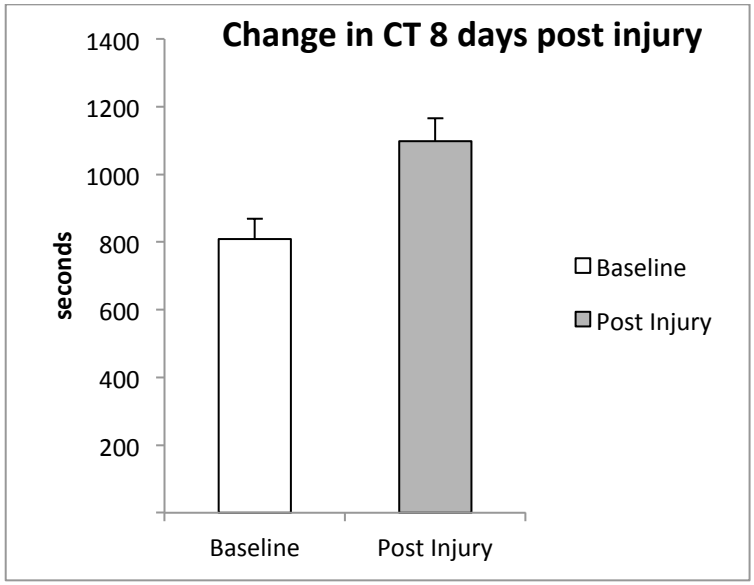


FIGURE 32

Effects of Time on Vertical Activity collapse across Blast, Stress, and Sex

$F(1,79)=6.23$, $p= .015$, $\eta^2= .073$

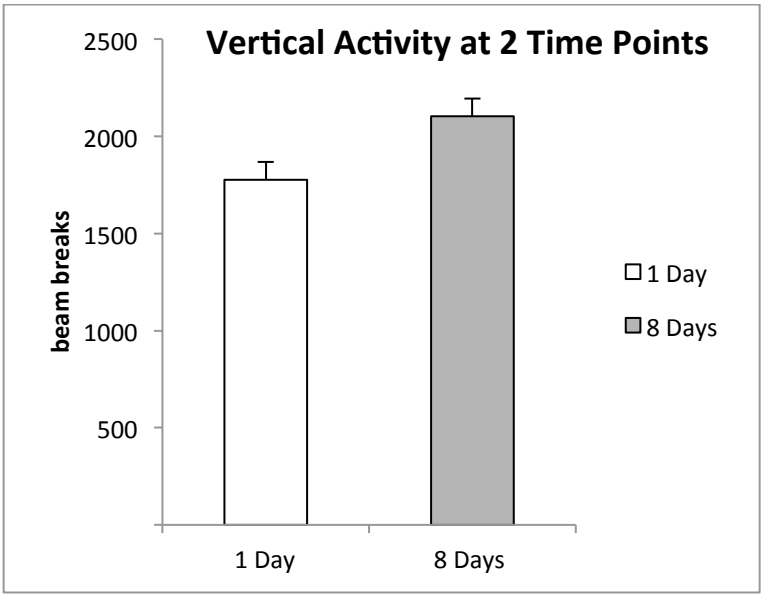


FIGURE 33

Effects of Blast and Stress on Vertical Activity one day after injury

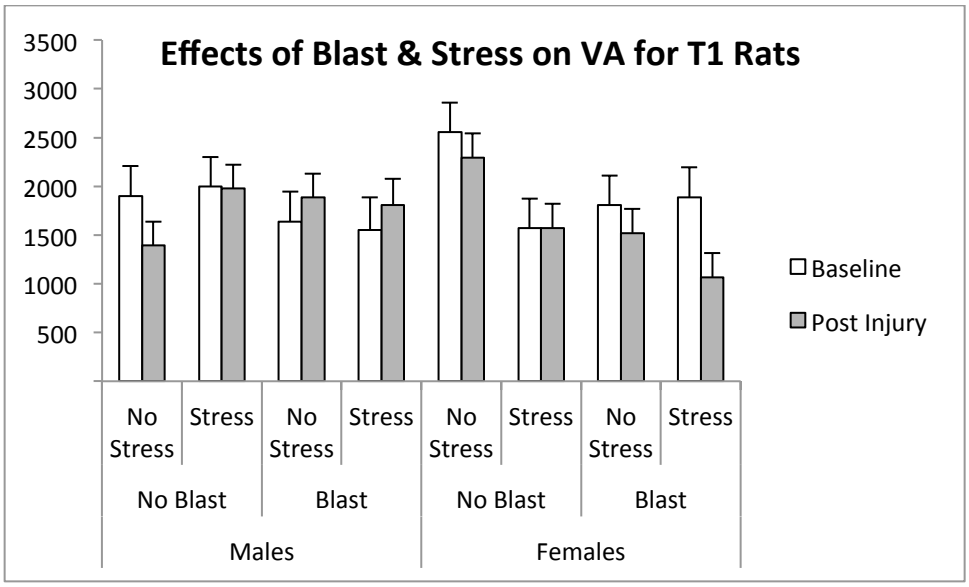


FIGURE 34

Effects of Stress on Vertical Activity one day after injury collapsed across Blast

$F(1,40)= 4.24$, $p=.046$, $\eta^2= .098$

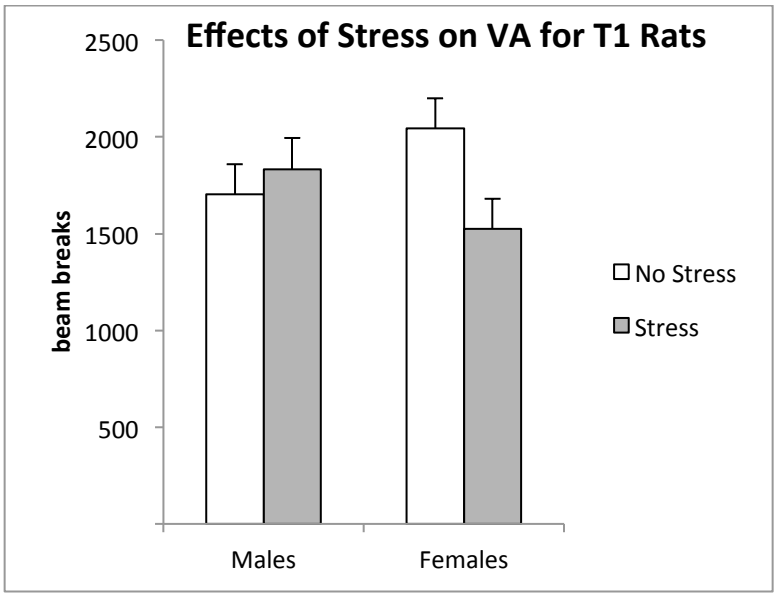


FIGURE 35

Effects of Blast on Vertical Activity for Stressed, Females one day after injury

$F(1,10)= 6.99$, $p= .025$, $\eta^2 = .411$

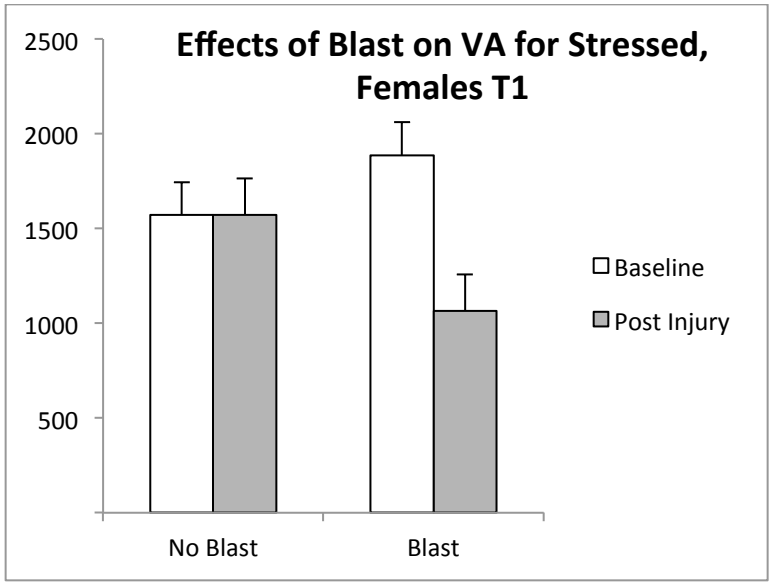


FIGURE 36

Effects of Blast and Stress on Vertical Activity eight days after injury

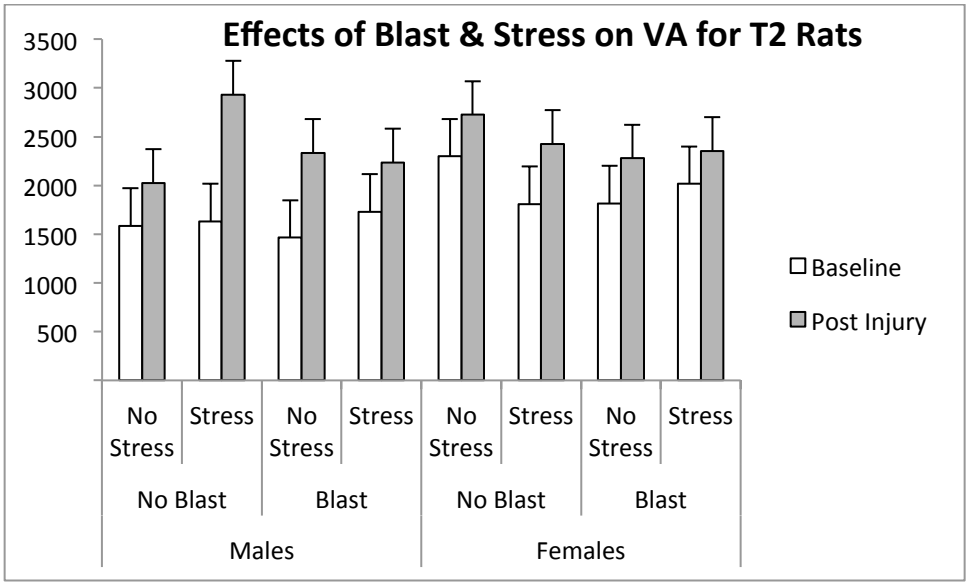
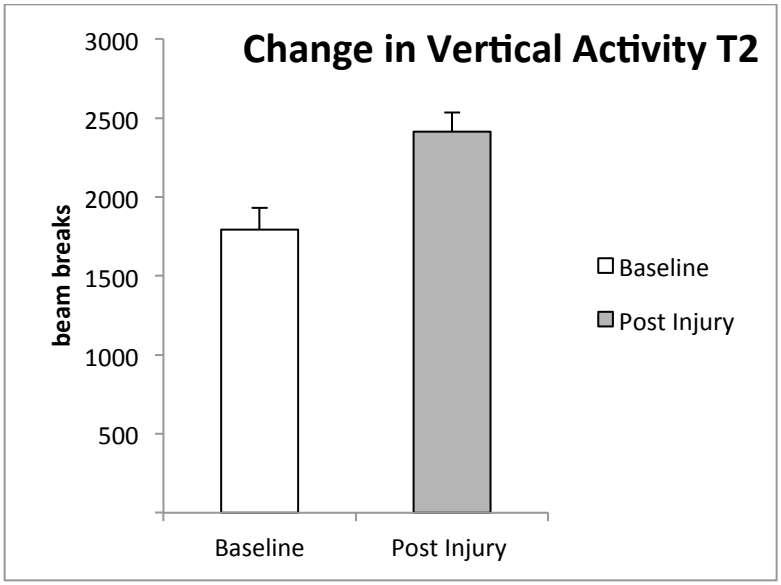


FIGURE 37

Change in Vertical Activity from baseline to eight days after injury

$F(1,40)= 16.16, p<.001, \eta^2 = .288$



APPENDIX B- TABLES

Table 1

EXPERIMENT'S SUBJECT BREAKDOWN (N=143)								
	MALE (n=79)				FEMALE (n=64)			
	NO STRESS (n= 40)		STRESS (n=39)		NO STRESS (n=32)		STRESS (n=32)	
	T1 (n=24)	T2 (n=16)	T1 (n=23)	T2 (n=16)	T1 (n=16)	T2 (n=16)	T1 (n=16)	T2 (n=16)
CONTROL (n=72)	12	8	12	8	8	8	8	8
BOP (n=71)	12	8	11	8	8	8	8	8

Table 2

Overall ANOVA of NSS-R change scores							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
INJURY	22.142	1	22.142	4.237	0.042	0.032	0.533
STRESS	1.929	1	1.929	0.369	0.545	0.003	0.093
SEX	0.313	1	0.313	0.060	0.807	0.000	0.057
TIME	3.089	1	3.089	0.591	0.443	0.005	0.119
INJURY * STRESS	0.001	1	0.001	0.000	0.987	0.000	0.050
INJURY * SEX	0.313	1	0.313	0.060	0.807	0.000	0.057
INJURY * TIME	0.007	1	0.007	0.001	0.970	0.000	0.050
STRESS * SEX	0.554	1	0.554	0.106	0.745	0.001	0.062
STRESS * TIME	5.273	1	5.273	1.009	0.317	0.008	0.169
SEX * TIME	4.136	1	4.136	0.791	0.375	0.006	0.143
INJURY * STRESS * SEX	29.137	1	29.137	5.575	0.020	0.042	0.649
INJURY * STRESS * TIME	0.109	1	0.109	0.021	0.885	0.000	0.052
INJURY * SEX * TIME	4.136	1	4.136	0.791	0.375	0.006	0.143
STRESS * SEX * TIME	0.000	1	0.000	0.000	0.998	0.000	0.050
INJURY * STRESS * SEX * TIME	0.017	1	0.017	0.003	0.955	0.000	0.050
Error	663.702	127	5.226				
Total	857.750	143					

Table 3

Male Time 1 ANOVA NSS-R change scores							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
INJURY	0.947	1	0.947	0.149	0.701	0.003	0.067
STRESS	1.051	1	1.051	0.166	0.686	0.004	0.068
INJURY * STRESS	21.940	1	21.940	3.459	0.070	0.074	0.444
Error	272.720	43	6.342				
Total	385.250	47					

Table 4

Female Time 1 ANOVA NSS-R change scores							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
INJURY	28.125	1	28.125	5.046	0.033	0.153	0.583
STRESS	0.031	1	0.031	0.006	0.941	0.000	0.051
INJURY * STRESS	0.500	1	0.500	0.090	0.767	0.003	0.060
Error	156.063	28	5.574				
Total	196.000	32					

Table 5

Male Time 2 ANOVA NSS-R change scores							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
INJURY	2.531	1	2.531	0.787	0.383	0.027	0.137
STRESS	4.500	1	4.500	1.399	0.247	0.048	0.208
INJURY * STRESS	6.125	1	6.125	1.904	0.179	0.064	0.266
Error	90.063	28	3.217				
Total	114.500	32					

Table 6

Female Time 2 ANOVA NSS-R change scores							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
INJURY	9.031	1	9.031	2.104	0.158	0.070	0.288
STRESS	2.000	1	2.000	0.466	0.500	0.016	0.101
INJURY * STRESS	8.000	1	8.000	1.864	0.183	0.062	0.261
Error	120.188	28	4.292				
Total	162.000	32					

Table 7

Overall RMANOVA of Horizontal Activity- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	6577.72	1	6577.72	0.00	0.98	0.00	0.05
measure * INJURY	33405218.41	1	33405218.41	2.09	0.15	0.03	0.30
measure * STRESS	21598133.37	1	21598133.37	1.35	0.25	0.02	0.21
measure * SEX	10212491.90	1	10212491.90	0.64	0.43	0.01	0.12
measure * TIME	104663127.25	1	104663127.25	6.53	0.01	0.08	0.71
measure * INJURY * STRESS	57760409.52	1	57760409.52	3.61	0.06	0.04	0.47
measure * INJURY * SEX	27194288.05	1	27194288.05	1.70	0.20	0.02	0.25
measure * INJURY * TIME	12435052.58	1	12435052.58	0.78	0.38	0.01	0.14
measure * STRESS * SEX	120764473.01	1	120764473.01	7.54	0.01	0.09	0.77
measure * STRESS * TIME	4232011.40	1	4232011.40	0.26	0.61	0.00	0.08
measure * SEX * TIME	2014385.83	1	2014385.83	0.13	0.72	0.00	0.06
measure * INJURY * STRESS * SEX	1002362.47	1	1002362.47	0.06	0.80	0.00	0.06
measure * INJURY * STRESS * TIME	4005659.40	1	4005659.40	0.25	0.62	0.00	0.08
measure * INJURY * SEX * TIME	49622118.32	1	49622118.32	3.10	0.08	0.04	0.41
measure * STRESS * SEX * TIME	36062304.27	1	36062304.27	2.25	0.14	0.03	0.32
measure * INJURY * STRESS * SEX * TIME	177900.25	1	177900.25	0.01	0.92	0.00	0.05
Error(measure)	1265566867.73	79	16019833.77				

Table 8

Overall RMANOVA of Horizontal Actvity- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	60024967971.58	1	60024967971.58	1663.42	0.00	0.95	1.00
INJURY	25725200.36	1	25725200.36	0.71	0.40	0.01	0.13
STRESS	1937924.75	1	1937924.75	0.05	0.82	0.00	0.06
SEX	317685264.07	1	317685264.07	8.80	0.00	0.10	0.83
TIME	164771345.15	1	164771345.15	4.57	0.04	0.05	0.56
INJURY * STRESS	17803095.87	1	17803095.87	0.49	0.48	0.01	0.11
INJURY * SEX	95095.47	1	95095.47	0.00	0.96	0.00	0.05
INJURY * TIME	13351101.64	1	13351101.64	0.37	0.54	0.00	0.09
STRESS * SEX	114491659.95	1	114491659.95	3.17	0.08	0.04	0.42
STRESS * TIME	37345649.54	1	37345649.54	1.03	0.31	0.01	0.17
SEX * TIME	4094339.06	1	4094339.06	0.11	0.74	0.00	0.06
INJURY * STRESS * SEX	33739667.46	1	33739667.46	0.93	0.34	0.01	0.16
INJURY * STRESS * TIME	19637650.33	1	19637650.33	0.54	0.46	0.01	0.11
INJURY * SEX * TIME	17855669.70	1	17855669.70	0.49	0.48	0.01	0.11
STRESS * SEX * TIME	19436029.83	1	19436029.83	0.54	0.47	0.01	0.11
INJURY * STRESS * SEX * TIME	8479051.03	1	8479051.03	0.23	0.63	0.00	0.08
Error	2850733210.93	79	36085230.52				

Table 9

Overall RMANOVA of Center Time- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	472635.17	1	472635.17	3.38	0.07	0.04	0.44
measure * INJURY	5546.47	1	5546.47	0.04	0.84	0.00	0.05
measure * STRESS	5100.71	1	5100.71	0.04	0.85	0.00	0.05
measure * SEX	239052.27	1	239052.27	1.71	0.20	0.02	0.25
measure * TIME	1699639.26	1	1699639.26	12.14	0.00	0.13	0.93
measure * INJURY * STRESS	5062.26	1	5062.26	0.04	0.85	0.00	0.05
measure * INJURY * SEX	48633.09	1	48633.09	0.35	0.56	0.00	0.09
measure * INJURY * TIME	88840.06	1	88840.06	0.63	0.43	0.01	0.12
measure * STRESS * SEX	70338.85	1	70338.85	0.50	0.48	0.01	0.11
measure * STRESS * TIME	2452.06	1	2452.06	0.02	0.90	0.00	0.05
measure * SEX * TIME	333844.95	1	333844.95	2.38	0.13	0.03	0.33
measure * INJURY * STRESS * SEX	236.41	1	236.41	0.00	0.97	0.00	0.05
measure * INJURY * STRESS * TIME	35749.26	1	35749.26	0.26	0.61	0.00	0.08
measure * INJURY * SEX * TIME	205921.72	1	205921.72	1.47	0.23	0.02	0.22
measure * STRESS * SEX * TIME	114250.49	1	114250.49	0.82	0.37	0.01	0.15
measure * INJURY * STRESS * SEX * TIME	688.40	1	688.40	0.00	0.94	0.00	0.05
Error(measure)	11060608.94	79	140007.71				

Table 10

Overall RMANOVA of Center Time- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	141100095.31	1	141100095.31	586.26	0.00	0.88	1.00
INJURY	40000.92	1	40000.92	0.17	0.68	0.00	0.07
STRESS	259513.81	1	259513.81	1.08	0.30	0.01	0.18
SEX	1307601.56	1	1307601.56	5.43	0.02	0.06	0.63
TIME	1565110.86	1	1565110.86	6.50	0.01	0.08	0.71
INJURY * STRESS	776.88	1	776.88	0.00	0.95	0.00	0.05
INJURY * SEX	4897.87	1	4897.87	0.02	0.89	0.00	0.05
INJURY * TIME	131559.00	1	131559.00	0.55	0.46	0.01	0.11
STRESS * SEX	1375186.39	1	1375186.39	5.71	0.02	0.07	0.66
STRESS * TIME	99506.11	1	99506.11	0.41	0.52	0.01	0.10
SEX * TIME	7001.04	1	7001.04	0.03	0.87	0.00	0.05
INJURY * STRESS * SEX	226783.33	1	226783.33	0.94	0.33	0.01	0.16
INJURY * STRESS * TIME	57798.55	1	57798.55	0.24	0.63	0.00	0.08
INJURY * SEX * TIME	91278.20	1	91278.20	0.38	0.54	0.00	0.09
STRESS * SEX * TIME	376201.78	1	376201.78	1.56	0.21	0.02	0.23
INJURY * STRESS * SEX * TIME	10460.56	1	10460.56	0.04	0.84	0.00	0.05
Error	19013582.42	79	240678.26				

Table 11

Overall RMANOVA of Vertical Activity- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	2358844.63	1	2358844.63	5.12	0.03	0.06	0.61
measure * INJURY	34560.00	1	34560.00	0.08	0.78	0.00	0.06
measure * STRESS	114212.67	1	114212.67	0.25	0.62	0.00	0.08
measure * SEX	1256671.30	1	1256671.30	2.73	0.10	0.03	0.37
measure * TIME	7485171.38	1	7485171.38	16.26	0.00	0.17	0.98
measure * INJURY * STRESS	1477893.52	1	1477893.52	3.21	0.08	0.04	0.42
measure * INJURY * SEX	569725.19	1	569725.19	1.24	0.27	0.02	0.20
measure * INJURY * TIME	117139.84	1	117139.84	0.25	0.62	0.00	0.08
measure * STRESS * SEX	269935.88	1	269935.88	0.59	0.45	0.01	0.12
measure * STRESS * TIME	20741.07	1	20741.07	0.05	0.83	0.00	0.06
measure * SEX * TIME	766.44	1	766.44	0.00	0.97	0.00	0.05
measure * INJURY * STRESS * SEX	63375.00	1	63375.00	0.14	0.71	0.00	0.07
measure * INJURY * STRESS * TIME	13004.63	1	13004.63	0.03	0.87	0.00	0.05
measure * INJURY * SEX * TIME	735318.60	1	735318.60	1.60	0.21	0.02	0.24
measure * STRESS * SEX * TIME	21239.84	1	21239.84	0.05	0.83	0.00	0.06
measure * INJURY * STRESS * SEX * TIME	283670.14	1	283670.14	0.62	0.43	0.01	0.12
Error(measure)	36374540.50	79	460437.22				

Table 12

Overall RMANOVA of Vertical Actvity- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	713403461.87	1	713403461.87	877.65	0.00	0.92	1.00
INJURY	2014385.83	1	2014385.83	2.48	0.12	0.03	0.34
STRESS	170477.09	1	170477.09	0.21	0.65	0.00	0.07
SEX	670255.45	1	670255.45	0.82	0.37	0.01	0.15
TIME	5071423.05	1	5071423.05	6.24	0.01	0.07	0.69
INJURY * STRESS	110806.26	1	110806.26	0.14	0.71	0.00	0.07
INJURY * SEX	543951.66	1	543951.66	0.67	0.42	0.01	0.13
INJURY * TIME	147169.04	1	147169.04	0.18	0.67	0.00	0.07
STRESS * SEX	3313291.11	1	3313291.11	4.08	0.05	0.05	0.51
STRESS * TIME	867309.83	1	867309.83	1.07	0.30	0.01	0.18
SEX * TIME	503881.52	1	503881.52	0.62	0.43	0.01	0.12
INJURY * STRESS * SEX	2991376.44	1	2991376.44	3.68	0.06	0.04	0.47
INJURY * STRESS * TIME	7128.60	1	7128.60	0.01	0.93	0.00	0.05
INJURY * SEX * TIME	164512.79	1	164512.79	0.20	0.65	0.00	0.07
STRESS * SEX * TIME	171331.02	1	171331.02	0.21	0.65	0.00	0.07
INJURY * STRESS * SEX * TIME	20881.79	1	20881.79	0.03	0.87	0.00	0.05
Error	64215641.23	79	812856.22				

Table 13

Time One RMANOVA for Horizontal Activity- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	50877015.52	1	50877015.52	3.31	0.08	0.08	0.43
measure * INJURY	2507916.31	1	2507916.31	0.16	0.69	0.00	0.07
measure * STRESS	3313643.01	1	3313643.01	0.22	0.65	0.01	0.07
measure * SEX	10519197.76	1	10519197.76	0.68	0.41	0.02	0.13
measure * INJURY * STRESS	45531717.47	1	45531717.47	2.96	0.09	0.07	0.39
measure * INJURY * SEX	74226526.26	1	74226526.26	4.83	0.03	0.11	0.57
measure * STRESS * SEX	142645104.01	1	142645104.01	9.27	0.00	0.19	0.84
measure * INJURY * STRESS * SEX	165804.23	1	165804.23	0.01	0.92	0.00	0.05
Error(measure)	599904341.23	39	15382162.60				

Table 14

Time One RMANOVA for Horizontal Activity- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	26621311695.15	1	26621311695.15	762.89	0.00	0.95	1.00
INJURY	37606540.23	1	37606540.23	1.08	0.31	0.03	0.17
STRESS	27805742.05	1	27805742.05	0.80	0.38	0.02	0.14
SEX	123302178.66	1	123302178.66	3.53	0.07	0.08	0.45
INJURY * STRESS	22212.06	1	22212.06	0.00	0.98	0.00	0.05
INJURY * SEX	7578747.06	1	7578747.06	0.22	0.64	0.01	0.07
STRESS * SEX	112744632.59	1	112744632.59	3.23	0.08	0.08	0.42
INJURY * STRESS * SEX	37559571.15	1	37559571.15	1.08	0.31	0.03	0.17
Error	1360919986.77	39	34895384.28				

Table 15

Time Two RMANOVA for Horizontal Activity- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	53829135.38	1	53829135.38	3.23	0.08	0.07	0.42
measure * INJURY	43842660.17	1	43842660.17	2.63	0.11	0.06	0.35
measure * STRESS	22756537.50	1	22756537.50	1.37	0.25	0.03	0.21
measure * SEX	1597536.00	1	1597536.00	0.10	0.76	0.00	0.06
measure * INJURY * STRESS	15868134.38	1	15868134.38	0.95	0.33	0.02	0.16
measure * INJURY * SEX	1694422.04	1	1694422.04	0.10	0.75	0.00	0.06
measure * STRESS * SEX	12575880.38	1	12575880.38	0.76	0.39	0.02	0.14
measure * INJURY * STRESS * SEX	1025066.67	1	1025066.67	0.06	0.81	0.00	0.06
Error(measure)	665662526.50	40	16641563.16				

Table 16

Time Two RMANOVA for Horizontal Activity- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	33655266570.67	1	33655266570.67	903.61	0.00	0.96	1.00
INJURY	1018052.04	1	1018052.04	0.03	0.87	0.00	0.05
STRESS	11273733.38	1	11273733.38	0.30	0.59	0.01	0.08
SEX	199417115.04	1	199417115.04	5.35	0.03	0.12	0.62
INJURY * STRESS	37885988.17	1	37885988.17	1.02	0.32	0.02	0.17
INJURY * SEX	10406934.00	1	10406934.00	0.28	0.60	0.01	0.08
STRESS * SEX	20038537.50	1	20038537.50	0.54	0.47	0.01	0.11
INJURY * STRESS * SEX	4247892.04	1	4247892.04	0.11	0.74	0.00	0.06
Error	1489813224.17	40	37245330.60				

Table 17

Time One RMANOVA for Center Time- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	187546.60	1	187546.60	1.10	0.30	0.03	0.18
measure * INJURY	68544.96	1	68544.96	0.40	0.53	0.01	0.09
measure * STRESS	236.90	1	236.90	0.00	0.97	0.00	0.05
measure * SEX	562010.46	1	562010.46	3.30	0.08	0.08	0.43
measure * INJURY * STRESS	33445.44	1	33445.44	0.20	0.66	0.01	0.07
measure * INJURY * SEX	26872.62	1	26872.62	0.16	0.69	0.00	0.07
measure * STRESS * SEX	2617.24	1	2617.24	0.02	0.90	0.00	0.05
measure * INJURY * STRESS * SEX	58.27	1	58.27	0.00	0.99	0.00	0.05
Error(measure)	6638080.67	39	170207.20				

Table 18

Time One RMANOVA for Center Time- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	55783324.80	1	55783324.80	277.34	0.00	0.88	1.00
INJURY	156392.15	1	156392.15	0.78	0.38	0.02	0.14
STRESS	336057.12	1	336057.12	1.67	0.20	0.04	0.24
SEX	554772.73	1	554772.73	2.76	0.10	0.07	0.37
INJURY * STRESS	35549.77	1	35549.77	0.18	0.68	0.00	0.07
INJURY * SEX	26615.44	1	26615.44	0.13	0.72	0.00	0.06
STRESS * SEX	1575512.09	1	1575512.09	7.83	0.01	0.17	0.78
INJURY * STRESS * SEX	69063.25	1	69063.25	0.34	0.56	0.01	0.09
Error	7844363.26	39	201137.52				

Table 19

Time Two RMANOVA for Center Time- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	2007192.60	1	2007192.60	18.15	0.00	0.31	0.99
measure * INJURY	25307.77	1	25307.77	0.23	0.63	0.01	0.08
measure * STRESS	7404.35	1	7404.35	0.07	0.80	0.00	0.06
measure * SEX	3997.71	1	3997.71	0.04	0.85	0.00	0.05
measure * INJURY * STRESS	7040.09	1	7040.09	0.06	0.80	0.00	0.06
measure * INJURY * SEX	230192.30	1	230192.30	2.08	0.16	0.05	0.29
measure * STRESS * SEX	184214.04	1	184214.04	1.67	0.20	0.04	0.24
measure * INJURY * STRESS * SEX	876.65	1	876.65	0.01	0.93	0.00	0.05
Error(measure)	4422528.27	40	110563.21				

Table 20

Time Two RMANOVA for Center Time- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	87270613.33	1	87270613.33	312.54	0.00	0.89	1.00
INJURY	13402.46	1	13402.46	0.05	0.83	0.00	0.06
STRESS	19049.12	1	19049.12	0.07	0.80	0.00	0.06
SEX	762393.08	1	762393.08	2.73	0.11	0.06	0.36
INJURY * STRESS	22869.11	1	22869.11	0.08	0.78	0.00	0.06
INJURY * SEX	70097.45	1	70097.45	0.25	0.62	0.01	0.08
STRESS * SEX	158380.63	1	158380.63	0.57	0.46	0.01	0.11
INJURY * STRESS * SEX	169419.61	1	169419.61	0.61	0.44	0.01	0.12
Error	11169219.16	40	279230.48				

Table 21

Time One RMANOVA for Vertical Activity- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	711280.04	1	711280.04	2.05	0.16	0.05	0.29
measure * INJURY	12074.19	1	12074.19	0.03	0.85	0.00	0.05
measure * STRESS	18576.26	1	18576.26	0.05	0.82	0.00	0.06
measure * SEX	651707.97	1	651707.97	1.88	0.18	0.05	0.27
measure * INJURY * STRESS	599414.63	1	599414.63	1.73	0.20	0.04	0.25
measure * INJURY * SEX	1283918.70	1	1283918.70	3.71	0.06	0.09	0.47
measure * STRESS * SEX	218608.17	1	218608.17	0.63	0.43	0.02	0.12
measure * INJURY * STRESS * SEX	38961.02	1	38961.02	0.11	0.74	0.00	0.06
Error(measure)	13501955.50	39	346203.99				

Table 22

Time One RMANOVA for Vertical Activity- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Powera
Intercept	295440459.45	1	295440459.45	507.51	0.00	0.93	1.00
INJURY	1605434.28	1	1605434.28	2.76	0.10	0.07	0.37
STRESS	892397.28	1	892397.28	1.53	0.22	0.04	0.23
SEX	5851.40	1	5851.40	0.01	0.92	0.00	0.05
INJURY * STRESS	86010.62	1	86010.62	0.15	0.70	0.00	0.07
INJURY * SEX	645408.01	1	645408.01	1.11	0.30	0.03	0.18
STRESS * SEX	2465313.57	1	2465313.57	4.23	0.05	0.10	0.52
INJURY * STRESS * SEX	1734644.30	1	1734644.30	2.98	0.09	0.07	0.39
Error	22703386.23	39	582138.11				

Table 23

Time Two RMANOVA for Vertical Activity- Within-Subject							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
measure	9238004.17	1	9238004.17	16.16	0.00	0.29	0.98
measure * INJURY	141220.04	1	141220.04	0.25	0.62	0.01	0.08
measure * STRESS	117600.00	1	117600.00	0.21	0.65	0.01	0.07
measure * SEX	605155.04	1	605155.04	1.06	0.31	0.03	0.17
measure * INJURY * STRESS	895134.38	1	895134.38	1.57	0.22	0.04	0.23
measure * INJURY * SEX	5340.17	1	5340.17	0.01	0.92	0.00	0.05
measure * STRESS * SEX	70742.04	1	70742.04	0.12	0.73	0.00	0.06
measure * INJURY * STRESS * SEX	311448.17	1	311448.17	0.54	0.46	0.01	0.11
Error(measure)	22872585.00	40	571814.63				

Table 24

Time Two RMANOVA for Vertical Activity- Between-Subjects							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	424629350.04	1	424629350.04	409.16	0.00	0.91	1.00
INJURY	543004.17	1	543004.17	0.52	0.47	0.01	0.11
STRESS	136052.04	1	136052.04	0.13	0.72	0.00	0.06
SEX	1182816.00	1	1182816.00	1.14	0.29	0.03	0.18
INJURY * STRESS	31248.17	1	31248.17	0.03	0.86	0.00	0.05
INJURY * SEX	55777.04	1	55777.04	0.05	0.82	0.00	0.06
STRESS * SEX	1001233.50	1	1001233.50	0.96	0.33	0.02	0.16
INJURY * STRESS * SEX	1271901.04	1	1271901.04	1.23	0.27	0.03	0.19
Error	41512255.00	40	1037806.38				

References

Acri, J.B., Grunberg, N.E., & Morse, D.E. (1991). The effects of nicotine on the acoustic startle reflex amplitude in rats. *Psychopharmacology, 104*, 244-248.

Allison, P.D., (1990). Change scores as dependent variables in regression analysis. *Sociological Methodology, 20*, 93-114.

Arfanakis, K., Haughton, V.M., Carew, J.D., Rogers, B.P., Dempsey, R.J., & Meyerand, M.E. (2002). Diffusion tensor MR imaging in diffuse axonal injury. *American Journal of Neuroradiology, 23*, 794‑802.

Armed Forces Health Surveillance Center. (2011). Retrieved from Armed Forces Health Surveillance Center website: http://www.afhsc.mil/home.

Axelsson, H., Hjelmqvist, H., Medin, A., Persson, J.K., & Suneson, A. (2000). Physiological changes in pigs exposed to a blast wave from a detonating high-explosive charge. *Military Medicine, 165*, 119–26.

Baum, A., Gatchel, R., & Krantz, D.S. (1997). *Introduction to health psychology*. 3rd ed. New York: McGraw-Hill.

Baum, A., & Singer, J.E. (Eds.). (1987). *Handbook of Psychology, Volume V, Stress*. Hillsdale, NJ: Lawrence Erlbaum Associates, Inc.

Bauman, R.A., Elsayed, N., Petras, J.M., & Widholm, J. (1997). Exposure to sublethal blast overpressure reduces the food intake and exercise performance of rats. *Toxicology, 121*, 65–79.

Bauman, R.A., Ling, G., Tong, L., Januszkiewicz, A., Agoston, D., Delanerolle, N., Kim, Y., Ritzel, D., Bell, R., Ecklund, J., Armonda, R., Bandak, F., & Parks, S. (2009) An introductory characterization of a combat-casualty-care relevant swine model of closed head injury resulting from exposure to explosive blast. *Journal of Neurotrauma, 26*, 841–60.

Beaumont, A., Marmarou, A., Hayasaki, K., Barzo, P., Fatouros, P., Corwin, F., Marmarou, C., & Dunbar, J. (2000). The permissive nature of blood brain barrier (BBB) opening in edema formation following traumatic brain injury. *Acta Neurochirurgica Supplementum*, *76,* 125–129.

Belanger, H.G., Kretzmer, T., Vanderploeg, R.D., & French, L.M. (2010). Symptom complaints following combat-related traumatic brain injury: relationship to traumatic brain injury severity and posttraumatic stress disorder. *Journal of International Neuropsychological Society, 16(1),* 194-199.

Belanger, H.G., Proctor-Weber, Z., Kretzmer, T., Kim, M., French, L.M., & Vanderploeg, R.D. (2011). Symptom complaints following reports of blast versus non-blast mild TBI: does mechanism of injury matter? *Clinical Neuropsychologist, 25(5*), 702-15.

Belzung, C., El Hage, W., Moindrot, N. & Griebel, G. (2001). Behavioral and neurochemical changes following predatory stress in mice. *Neuropharmacology, 41*(3), 400-408.

Berger, S.S. (2009). *Behavioral and biological effects of prenatal stress and social enrichment: Relevance to heart disease.* Uniformed Services University of the Health Sciences, Bethesda, MD.

Barry, D.M. (2011, November). Personal Communication about mbTBI to A.M. Yarnell, Bethesda, MD.

Bogo, V., Hutton, R.A., & Bruner, A. (1971). The effects of airblast on discriminated avoidance behavior in rhesus monkeys. In: *Technical Progress Report on Contract* (No. DA-49-146-XZ-372) Vol. DASA 2659. Washington, DC: Defense Nuclear Agency, 1–32.

Bowen, D.J., Eury, S.E., & Grunberg, N.E. (1986). Nicotine's effects on female rats' body weight: caloric intake and physical activity. *Pharmacology Biochemistry and Behavior,25(6),* 1131-1136.

Campbell, T., Lin, S., DeVries, C., & Lambert, K. (2003). Coping strategies in male and female rats exposed to multiple stressors. *Physiology & Behavior, 78*(3), 495-504.

Cannon, W.B. (1929). Organization of the physiologic homeostasis. *Psychological Reviews, 9,* 399-427.

Cannon, W.B. (1935). Stress and the environment. *American Journal of Medical Science, 189(1),* 1-4.

Carlezon, W. A., Pliakas, A. M., Parow, A. M., Detke, M. J., Cohen, B. M., & Renshaw, P. F. (2002). Antidepressant-like effects of cytidine in the forced swim test in rats. *Biology & Psychiatry, 51(11),* 882-889.

Center for Disease Control. (2010). Retrieved from Center for Disease Control website: www.cdc.gov.

Cernak, I., Wang, Z., Jiang, J., Bian, X. & Savic, J. (2001a). Cognitive deficits following blast injury-induced neurotrauma: possible involvement of nitric oxide. *Brain Injury, 15*, 593-612.

Cernak, I., Wang, Z., Jiang, J., Bian, X., & Savic, J. (2001b). Ultrastructural and functional characteristics of blast injury-induced neurotrauma. *Journal of Trauma, 50*, 695-706.

Cernak, I., Savic, J., Stanimirovic, D., Djuricic, B., & Mrsulja, B.B. (1990). The pathogenesis of pulmonary edema in sheep exposed to FAE charge. *Journal of Trauma(China),6(Suppl),* 267–271.

Cernak, I., Ignjatovic, D., Andelic, G., Savic, J. (1991). Metabolic changes as part of the general response of the body to the effect of blast waves. *Vojnosanit Pregl, 48*, 515–22.

Cernak, I., Savic, J., Malicevic, Z., Zunic, G., Radosevic, P., Ivanovic, I, & Davidovic, L. (1996). Involvement of the central nervous system in the general response to pulmonary blast injury. *Journal of Trauma, 40,* S100 –S104.

Cernak, I. (2005). Animal models of head trauma. *NeuroRx, 2*, 410-422.

Cernak, I., & Noble-Haeusslein, L.J. (2010). Traumatic brain injury: an overview of patho-biology with emphasis on military populations. *Journal of Cerebral Blood Flow and Metabolism, 30*, 255–66.

Chavko, M., Koller, W.A., Prusaczyk, W.K., & McCarron, R.M. (2007). Measurement of blast wave by a miniature fiber optic pressure transducer in the rat brain. *Journal of Neuroscience Methods, 159(2),* 277-281.

Chavko, Watanabe, T., Adeeb, S., Lankasky, J., Ahlers, S.T., & McCarron, R.M. (2011). Relationship between orientation to a blast and pressure wave propagation inside the rat brain. *Journal of Neuroscience Methods, 195*, 61-66.

Chen, J-K., Johnston, K.M., Collie, A., McCrory, P., & Ptito, A. (2007). A validation of the post concussion symptom scale in the assessment of complex concussion using cognitive testing and functional MRI. *Journal of Neurology, Neurosurgery, and Psychiatry, 78(11),*1231–1238.

Cherian, L., Robertson, C.S., Contant, C.F., & Bryan, R.M. (1994). Lateral cortical impact injury in rats: cerebrovascular effects of varying depth of cortical deformation and impact velocity. *Journal of Neurotrauma, 11,* 573–585.

Chiarelli, P.W. (2011, September). Opening keynote address presented at Uniformed Services University- Henry Jackson Foundation Military Medicine Symposium: The TBI spectrum, Washington, D.C.

Cicerone, K.D., & Kalmar, K. (1995). Persistent postconcussion syndrome: the structure of subjective complaints after mild traumatic brain injury. *Journal of Head Trauma Rehabilitation, 10*, 1–17.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24(4),* 385-396.

Cole, J.T., Yarnell, A.M., Kean, W.S., Gold, E., Lewis, B., Ren, M., McMullen, D.C., Jacobowitz, D., Pollard, H.B., O'Neill, J.T., Grunberg, N.E., Dalgard, C.L., Frank, J,. & Watson, W.D. (2011). Craniotomy: True sham for traumatic brain injury, or a sham of a sham? *Journal of Neurotrauma*, *28(3),*359-69.

Committee for the update of the guide for the care and use of laboratory animals. (2011). *Guide for Care and Use of Laboratory Animals, 8th edition*. Retrieved from National Institute of Health website: http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-Laboratory-animals.pdf.

Davey, G (Ed.). (1983). Editor's preface. In G. Davey (Ed.), *Animal models of human behavior: conceptual, evolutionary, and neurobiological perspectives* (pp. ix-xi). London: John Wiley & Sons.

Defense Centers of Excellence (DCoE) for Psychological Health and Traumatic Brain Injury and the Defense Veterans Brain Injury Center (DVBIC). (2009). *Traumatic brain injury care in the Department of Defense*. Retrieved from DCoE website: http://www.dcoe.health.mil/Content/Navigation/Documents/Traumatic%20Brain%20Injury%20Care%20in%20the%20Department%20of%20Defense.pdf.

Defense Veterans Brain Injury Center (DVBIC). (2011). Retrieved from DVBIC website: http://www.dvbic.org.

Detke, M. J., Rickels, M., & Lucki, I. (1995). Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology, 121(1),* 66-72.

Dixon, C.E., Clifton, G.L., Lighthall, J.W., Yaghmai, A.A., & Hayes, R.L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *Journal of Neuroscience Methods, 39,* 253–262.

Dozier, M., & Peloso, E. (2006). The role of early stressors in child health and mental health outcomes. *Archives of Pediatric and Adolescent Medicine, 160(12),* 1300-1301.

Dubrovina, N.I. (2006). Effects of activation of D1 dopamine receptors on extinction of a conditioned passive avoidance reflex and amnesia in aggressive and submissive mice. *Neuroscience and Behavioral Physiology*, *36(6)*, 679-684.

Elliot, B. (2004). Environmental enrichment, performance, and brain injury in male and female rats. Uniformed Services University of the Health Sciences, Bethesda, MD.

Elliott, B.M., Faraday, M.M., Phillips, J.M., & Grunberg, N.E. (2004). Effects of nicotine on elevated plus maze and locomotor activity in male and female adolescent and adult rats. *Pharmacology Biochemistry and Behavior, 77(1),* 21-28.

Elliott, B.M., & Grunberg, N.E. (2005). Effects of social and physical enrichment on open field activity differ in male and female Sprague-Dawley rats. *Behavioural Brain Research 165(2),* 187-196.

Elsayed, N. M. (1997). Toxicology of blast overpressure. *Toxicology, 121,* 1-15.

Emanuelson, I., Andersson-Holmkvist, R., Bjorklund, D., & Stalhammar, D. (2003). Quality of life and post-concussion symptoms in adults after mild traumatic brain injury: a population-based study in western Sweden. *Acta Neurologica Scandinavic, 108,* 332-338.

Endres, T., Apfelbach, R., & Fendt, M. (2005). Behavioral changes induced in rats by exposure to trimethylthiazoline, a component of fox odor. *Behavioral Neuroscience, 119*(4), 1004-1010.

Faraday, M.M. (2005). Stress revisited: a methodological and conceptual history. In: S. Yehuda, & D. Mustofsky D (Eds.). *Nutrients, Stress, and Medical Disorders* (pp. 3-20). Totowa, NJ: Humana Press.

Faraday, M.M., Blakeman, K.H., & Grunberg, N.E. (2005). Strain and sex alter effects of stress and nicotine on feeding, body weight, and HPA axis hormones. *Pharmacology Biochemistry & Behavior, 80*(4)*,* 577-589.

Faraday, M.M., Elliott, B.M., Phillips, J.M., & Grunberg, N.E. (2003). Adolescent and adult male rats differ in sensitivity to nicotine's activity effects. *Pharmacology Biochemistry and Behavior, 74(4),* 917-931.

Faraday, M.M., & Grunberg, N.E. (1999). The role of biobehavioral animal models in nicotine and tobacco research. *Society for Research on Nicotine and Tobacco Newsletter, 5(1),* 7-10.

Faraday, M.M., O'Donoghue, V.A., & Grunberg, N.E. (1999). Effects of nicotine and stress on startle amplitude and sensory-gating depend on rat strain and sex. *Pharmacology Biochemistry and Behavior, 62*(2), 273-284.

Faraday, M.M., O'Donoghue, V.A., & Grunberg, N.E. (2003). Effects of nicotine and stress on locomotion in Sprague-Dawley and Long-Evans male and female rats. *Pharmacology Biochemistry & Behavior, 74*(2), 325-333.

Faraday, M.M., Scheufele, P.M., Rahman, M.A., & Grunberg, N.E. (1999). Effects of chronic nicotine administration on locomotion depend on rat sex and housing condition. *Nicotine & Tobacco Research, 1(2),* 143-151.

Foda, M.A., & Marmarou, A. (1994). A new model of diffuse brain injury in rats. Part II: Morphological characterization. *Journal of Neurosurgery, 80,* 301–313.

Fourtassia, M., Hajjiouia, A., El Ouahabib, A., Benmassaouda, H., Hajjaj-Hassounia, N., & El Khamlichib. (2011). Long term outcome following mild traumatic brain injury in Moroccan patients. *Clinical Neurology and Neurosurgery, Epub ahead of print*.

Fride,E., Dan, Y., Feldon, J., Halevy, G., & Weinstock, M. (1986). Effects of prenatal stress on vulnerability to stress in prepubertal and adult rats. *Physiology and Behavior, 37(5),* 681-687.

Gonzalez Jatuff, A.S., Berastegui, M., Rodriguez, C.I., & Rodriguez Echandia, E.L. (1999). Permanent and transient effects of repeated preweaning stress on social and sexual behaviors of rats. *Stress, 3(2),* 97-106.

Grippo, A.J., Beltz, T.G., & Johnson, A.K. (2003). Behavioral and cardiovascular changes in the chronic mild stress model of depression. Physiology & Behavior, 78(4-5), 703-710.

Grunberg, N.E., & Bowen, D.J. (1985). The role of physical activity in nicotine's effects on body weight. *Pharmacology Biochemistry and Behavior, 23(5),* 851-854.

Grunberg, N.E., & Faraday, M.M. (2000). The value of animal models to examine the Gateway Hypothesis. In D. Kandel., et al. (Eds.) *Stages and Pathways of Involvement in Drug Use: Examining the Gateway Hypothesis*. New York: Cambridge University Press.

Grunberg, N. E., Yarnell, A.M., Chwa, A., Hutchison, M.A., Barry, E.S. (2011). A revised neurological severity scale (NSS-R and mNSS-R) for rodents. Society for Neuroscience, Washington, D.C.

Hamilton, K.R. (2010). Impulsive action, psychological stress, and behavioral sensitization to nicotine in a rat model of impulsivity. Uniformed Services University of the Health Sciences, Bethesda, MD.

Hamm, R. J. (2001). Neurobehavioral assessment of outcome following traumatic brain injury in rats: an evaluation of selected measures. *Journal of Neurotrauma, 18(11),* 1207-1216.

Hayley, S., Borowski, T., Merali, Z., & Anisman, H. (2001). Central monoamine activity in genetically distinct strains of mice following a psychogenic stressor: effects of predator exposure. *Brain Research, 892(2),* 293-300.

Heim, C., & Nemeroff, C.B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological Psychiatry, 49(12),* 1023-1039.

Hoge, C.W., Auchterlonie, J.L., & Milliken, C.S. (2006). Mental health problems, use of mental health services, and attrition from military service after returning from deployment to Iraq or Afghanistan. *JAMA, 295(9),*1023-1032.

Hoge, C.W., Castro, C.A., Messer, S.C., McGurk, D., Cotting, D.I., & Koffman, R.L. (2004). Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. *The New England Journal of Medicine, 351(1*), 13-22.

Hoge, C.W., McGurk, D., Thomas, J.L., Cox, A.L., Engel, C.C., & Castro, C.A. (2008). Mild traumatic brain injury in U.S. soldiers returning from Iraq. *New England Journal of Medicine, 358(5*), 453-463.

Iverson, K.M., Hendricks, A.M., Kimerling, R., Krengel, M., Meterko, M., Stolzmann, K.L., Baker, E., Pogoda, T.K., Vasterling, J.L., & Lew, H.L. (2011). Psychiatric Diagnoses and Neurobehavioral Symptom Severity among OEF/OIF VA Patients with Deployment-Related Traumatic Brain Injury: A Gender Comparison. *Women's Health Issues, 21-4S*, S210– S217.

Jacob, H.J. (1999). Functional genomics and rat models. *Genome Research, 9(11),* 1013-1016.

Jaffin, J.H,, McKinney, L., Kinney, R.C., Cunningham, J.A., Moritz, D.M., Kraimer, J.M., Graeber, G.M., Moe, J.B., Salanderm J.M., & Harmon, J.W. (1987). A laboratory model for studying blast overpressure injury. *Journal of Trauma, 27*, 349–56.

Joint Mental Health Advisory Team 7 (J-MHAT 7). (2011). *Joint Mental Health Advisory Team 7 Operation Enduring Freedom 2010 Afghanistan*. Retrieved from U.S. Army Medicine website: http://www.armymedicine.army.mil/reports/mhat/mhat_vii/J_MHAT_7.pdf.

Jones, N.C., Cardamone, L., Williams, J.P., Salzberg, M.R., Myers, D., & O'Brien, T.J. (2008). Experimental traumatic brain injury induces a pervasive hyperanxious phenotype in rats. *Journal of Neurotrauma, 25(11),* 1367-1374.

Jorge, R., & Robinson, R.G. (2002). Mood disorders following traumatic brain injury. *NeuroRehabilitation, 17*, 311–324.

Jorge, R.E., Robinson, R.G. & Arndt, S.V. (1993). Are depressive symptoms specific for a depressed mood in traumatic brain injury? *The Journal of Nervous and Mental Disease,181,* 91–99.

Jorge, R.E., Robinson, R.G., Moser, D., Tateno, A., Crespo-Facorro, B., & Arndt, S. (2004). Major depression following traumatic brain injury. *Archives of General Psychiatry, 61,*42-50.

Kamnaksh, A., Kovesdi, E., Kwon, S-K., Wingo, D., Ahmed, F., Grunberg, N., Long, J., & Agoston, D. (2011). Factors affecting blast traumatic brain injury. *Journal of Neurotrauma, 28(10),* 2145-2153.

Kazanis, I. (2005). CNS injury research; reviewing the last decade: methodological errors and a proposal for a new strategy. *Brain Research Reviews, 50*, 377–86.

Klonoff, H. (1971). Head injuries in children: predisposing factors, accident conditions, accident proneness, and sequelae. *American Journal of Public Health, 61*, 2405–2417.

Kovesdi. E., Gyorgy, A.B., Kwon, S.K., Wingo, D.L., Kamnaksh, A., Long, J.B., Kasper, C.E., & Agoston, D.V. (2011). The effect of enriched environment on the outcome of traumatic brain injury: a behavioral, proteomics, and histological study. *Frontiers in Neuroscience, 5(42)*.

Kwon, S.K., Kovesdi, E., Gyorgy, A.B., Wingo, D., Kamnaksh, A., Walker, J., Long, J.B. & Agoston, D.V. (2011). Stress and traumatic brain injury: a behavioral, proteomics, and histological study. *Frontiers in Neurology, 2(12).*

LaPlaca, M.C., Simon, C.M., Prado, G.R., & Cullen, D.K. (2007). CNS injury biomechanics and experimental models. *Progress in Brain Research, 161,* 13–26.

Lazarus, R.S. (1966). *Psychological stress and the coping process*. New York: McGraw-Hill.

Lew, H.L., Otis, J.D., Tun, C., Kerns, R.D., Clark, M.E., & Cifu, D.X. (2009). Prevalence of chronic pain, posttraumatic stress disorder, and persistent postconcussive symptoms in OIF/OEF veterans: polytrauma clinical triad. *Journal of Rehabilitation Research & Development, 46(6),* 697-702.

Lew, H.L., Pogoda, T.K., Baker, E., Stolzmann, K.L., Meterko, M., Cifu, D.X., Amara, J., & Hendricks, A.M. (2011). Prevalence of dual sensory impairment and its association with traumatic brain injury and blast exposure in OEF/OIF Veterans. *Journal of Head Trauma and Rehabilitation, Epub ahead of print*.

Lewis, L., & Rosenberg, S.J. (1990). Psychoanalytic psychotherapy with brain-injured adult psychiatric patients. *Journal of Nervous and Mental Disease*, *178*, 69–77.

Ling, G., Bandak, F., Armonda, R., Grant, G., & Ecklund, J. (2009). Explosive blast neurotrauma. *Journal of Neurotrauma, 26*, 815-825.

Ling, G.S.F., Lee, E.Y., & Kalehua, A.N. (2004). Traumatic brain injury in the rat using the fluid- percussion model. *Current Protocols in Neuroscience,* 9.2.1-9.2.11.

Long, J.B., Bentley, T.L., Wessner, K.A., Cerone, C., Sweeney, S., & Bauman, R.A. (2009). Blast overpressure in rats: recreating a battlefield injury in the laboratory. *Journal of Neurotrauma, 26,* 827-840.

Long, S.M. (2010). Effects of exercise training and social enrichment on stress resilience in male and female Long-Evans rats. Uniformed Services University of the Health Sciences, Bethesda, MD.

Mahmood, A., Lu, D., Wang, L., Li, Y., Lu, M., & Chopp, M. (2001). Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. *Neurosurgery, 49(5),* 1196-1203.

Manvelyan, H. (2006). Contemporary experimental models of traumatic brain injury. *Georgian Med News, 140*, 1306.

Marmarou, A., Foda, M.A., van den Brink, W., Campbell, J., Kita, H., & Demetriadou, K. (1994). A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *Journal of Neurosurgery, 80,* 291–300.

Marti, M., Mela, F., Fantin, M., Zucchini, S., Brown, J.M., Witta, J., Di Benedetto, M., Buzas, B., Reinscheid, R.K., Salvadori, S., Guerrini, R., Romualdi, P., Candeletti, S., Simonato, M., Cox, B.M., & Morari, M. (2005). Blockade of nociceptin/orphanin FQ transmission attenuates symptoms and neurodegeneration associated with Parkinson's disease. *Journal of Neuroscience, 25(42),* 9591-9601.

Martin, E.M., Lu, W.C., Helmick, K., French, L., & Warden, D.L. (2008). Traumatic brain injuries sustained in the Afghanistan and Iraq wars. *American Journal of Nursing, 108,*40–47.

Masini, C.V., Sauer, S., & Campeau, S. (2005). Ferret odor as a processive stress model in rats: neurochemical, behavioral, and endocrine evidence. *Behavioral Neuroscience, 119*(1), 280-292.

Mason, J.W. (1975). A historical view of the stress field. *Journal of Human Stress,* 22-36.

Mayorga, M. A. (1997). The pathology of primary blast overpressure injury. *Toxicology, 121*,17-28.

McEwen, B.S. (2004). Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Annals of the New York Academy of Sciences, 1032,* 1-7.

McIntosh, T.K., Noble, L., Andrews, B., & Faden, A.I. (1987) Traumatic brain injury in the rat: characterization of a midline fluid-percussion model. *Central Nervous System Trauma, 4,*119 –134.

Mechiel Korte, S., & De Boer, S.F. (2003). A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. *European Journal of Pharmacology, 463*(1-3), 163-175.

Mittl, R.L., Grossman, R.I., Hiehle, J.F., Hurst, R.W., Kauder, D.R., Gennarelli, T.A., & Alburger, G.W. (1994). Prevalence of MR evidence of diffuse axonal injury in patients with mild head injury and normal head CT findings. *American Journal of Neuroradiology, 15*, 1583‑1589.

Moochhala, S.M., Md, S., Lu, J., Teng, C.H., & Greengrass, C. (2004). Neuroprotective role of aminoguanidine in behavioral changes after blast injury. *Journal of Trauma, 56*, 393–403.

Morales, D.M., Marklund, N., Lebold, D., Thompson, H.J., Pitkanen, A., Maxwell, W.L., Longhi, L., Laurer, H., Maegele, M., Neugebauer, E., Graham, D.I., Stocchetti, N., & McIntosh, T.K. (2005). Experimental models of traumatic brain injury: do we really need to build a better mousetrap? *Neuroscience, 136*, 971–989.

Morse, D.E., Davis, H.D., Popke, E.J., Brown, K.J., O'Donoghue, V.A., & Grunberg, N.E. (1997). Effects of ddC and AZT on locomotion and acoustic startle. Acute effects in female rats. *Pharmacology Biochemistry and Behavior, 56(2),* 221-228.

Mundie, T.G., Dodd, K.T., Lagutchik, M.S., Morris, J.R., & Martin, D. (2000). Effects of blast exposure on exercise performance in sheep. *Journal of Trauma, 48*, 1115–1121.

Nampiaparampil, D.E. (2008). Prevalence of chronic pain after traumatic brain injury: a systematic review. *JAMA, 300(6),* 711-719.

Okie, S. (2005). Traumatic brain injury in the war zone. *New England Journal of Medicine, 352*, 2043-2047.

Ojeda, S.R., & Urbanski, H.F. (1994). Puberty in the rat. In E. Knobil & J.D. Neill (Eds.), *The physiology of reproduction. 2nd ed., Vol 2* (pp. 612-619). New York: Raven Press.

Patil, V.K., St. Andre, J.R., Crisan, E., Smith, B.M., Evans, C.T., Steiner, M.L., & Pape, T.L. (2011). Prevalence and treatment of headaches in veterans with mild traumatic brain injury. *Headache, 51(7),* 1112-1121.

Perry, M.E. (2009). Effects of acute and recurrent stress during adolescence on subsequent indices of adult behavioral health. Uniformed Services University of the Health Sciences, Bethesda, MD.

Petit-Demouliere, B., Chenu, F., & Bourin, M. (2005). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology, 177(3),* 245-255.

Phillips, Y., & Richmond, D. (1991). Primary blast injury and basic research: a brief history. In R. Bellamy and R. Zajtchuk (Eds.), *Conventional warfare: ballistic, blast and burn injuries*. Washington, DC: Office of the Surgeon General at Textbook of Military Medicine Publications, 5, 221–335.

Pliakas, A. M., Carlson, R. R., Neve, R. L., Konradi, C., Nestler, E. J., & Carlezon, W. A., Jr. (2001). Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *Journal of Neuroscience, 21(18),* 7397-7403.

Porsolt, R., Le Pichon, M., & Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*, *266,* 730-732.

Porterfield, V.M., Zimomra, Z.R., Caldwell, E.A., Camp, R.M., Gabella, K.M., & Johnson, J.D. (2011). Rat strain differences in restraint stress-induced brain cytokines. *Cognitive,Behavioral, and Systems Neuroscience, 188,* 48-54.

Potts, M.B., Adwanikar, H., & Noble-Haeusslein, L.J. (2009). Models of traumatic cerebellar injury. *Cerebellum, 8*, 211–221.

Rao, V., & Lyketsos, C. (2000). Neuropsychiatric sequelae of traumatic brain injury. *Psychosomatics 41(2)*, 92-103.

Raygada, M., Shaham, Y., Nespor, S.M., Kant, G.J., & Grunberg, N.E. (1992). Effect of stress on hypothalamic insulin in rats. *Brain Research Bulletin, 29*(1), 129-134.

Readnower, R.D., Chavko, M., Adeeb, S., Conroy, M.D., Pauly, J.R., McCarron, R.M., & Sullivan, P.G. (2010). Increase in blood-brain barrier permeability, oxidative stress, and active microglia in a rat model of blast-induced traumatic brain injury. *Journal of Neuroscience Research, 88,* 3530-3539.

Regan, T. (2004). Report: High survival rate for US troops wounded in Iraq. *Christian Science Monitor*, November 29, 2004.

Richmond, D.R., Damon, E.G., Bowen, I.G., Fletcher, E.R., & White, C.S. (1967). *Air-blast studies with eight species of mammals*. Technology Progress Report DASA 1854. Fission Product Inhalation Project, 1–44.

Rosenfeld, J.V., & Ford, N.L. (2010). Bomb blast, mild traumatic brain injury and psychiatric morbidity: a review. *Injury, 41(5),* 437-43.

Rosenzweig, M.R., & Bennett, E. (1996). Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behavioural Brain Research, 78*, 57-65.

Rosenweig, M.R., Krech, D., Bennett, E.L., & Diamond, M. (1962). Effects of environmental complexity and training on brain chemistry and anatomy. A replication and extension. *Journal of Cognitive and Physiological Psychology, 55*, 429-437.

Ruff, R.L, Ruff, S.S., & Wang, X. (2011). Neurological Deficits and Post Traumatic Stress Disorder (PTSD) Are Related to the Number of Episodes of Mild Traumatic Brain Injury in US Combat Veterans. Presented at the 63rd Annual Meeting of the American Academy of Neurology, Richmond, VA.

Saljo, A., Bao, F., Haglid, K.G., & Hansson, H.A. (2000) Blast exposure causes redistribution of phosphorylated neurofilament subunits in neurons of the adult rat brain. *Journal of Neurotrauma, 17*, 719–26.

Saljo, A., Bao, F., Hamberger, A., Haglid, K.G., & Hansson, H.A. (2001). Exposure to short-lasting impulse noise causes microglial and astroglial cell activation in the adult rat brain. *Pathophysiology, 8,* 105-111.

Saljo, A., Svensson, B., Mayorga, M., Hamberger, A., & Bolouri, H. (2009). Low levels of blast raises intracranial pressure and impairs cognitive function in rats. *Journal of Neurotrauma, 28*, 1345–1352.

Sarkisova, K.Y., Kulikov, M.A., Midzyanovskaya, I.S., & Folomkina, A.A. (2008). Dopamine-dependent nature of depression-like behavior in WAG/Rij rats with genetic absence epilepsy. *Neuroscience and Behavioral Physiology, 38(2),* 119-128.

Savic, J., Tatic, V., Ignjatovic, D., Mrda, V., Erdeljan, D., Cernak, I., Vujnov, S., Simovic, M., Andelic, G., & Duknic, M. (1991) Pathophysiologic reactions in sheep to blast waves from detonation of aerosol explosives. *Vojnosanit Pregl, 48*, 499–506.

Sayer, N. A., Rettmann, N. A., Carlson, K. F., Bernardy, N., Sigford, B. J., Hamblen, J. L., et al. (2009). Veterans with history of mild traumatic brain injury and posttraumatic stress disorder: Challenges from provider perspective. *Journal of Rehabilitation Research and Development, 46,* 703-715.

Schell, T. l., & Marshall, G. N. (2008). Survey of individuals previously deployed for OEF/OIF. In T.Tanielian & l. H. Jaycox (Eds.), *Invisible wounds of war: Psychological andcognitive injuries, their consequences, and services to assist recovery* (pp. 87-115). Santa Monica, CA: RAND Center for Military Health Policy Research.

Schmidt, R.H., & Grady, M.S. (1993). Regional patterns of blood-brain barrier breakdown following central and lateral fluid percussion injury in rodents. *Journal of Neurotrauma,10,* 415– 430.

Selye, H. (1956). Stress and psychobiology. *Journal of Clinical and Experimental Psychopathology, 17(4),* 370-375.

Selye, H. (1946). The general adaptation syndrome and the diseases of adaptation. *Journal of Clinical Endocrinology, 2,* 117-230.

Selye, H. (1973). The evolution of the stress concept. *American Scientist, 61,* 692-699.

Shafer, S.T. (2006). Behavioral and biological effects of housing conditions and stress in male rats: relevance to heart disease. Uniformed Services University of the Health Sciences, Bethesda, MD.

Shaham, Y., Klein, L.C., Alvares, K., & Grunberg, N.E. (1993). Effect of stress on oral fentanyl consumption in rats in an operant self-administration paradigm. *Pharmacology Biochemistry and Behavior, 46*, 315-322.

Shohami, E., Novikov, M., & Bass, R. (1995). Long-term effect of HU-211, a novel non-competitive NMDA antagonist, on motor and memory functions after closed head injury in the rat. *Brain Research, 674*(1), 55-62.

Spear, L.P., & Brake, S.C. (1983). Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. *Developmental Psychobiology, 16*, 83-109.

Starosciak, A. (2010). Effects of stress and social enrichment on alcohol intake, biological and psychological stress responses in rats. Uniformed Services University of the HealthSciences, Bethesda, MD.

Survivability/Vulnerability Information Analysis Center. (2009). *Screening and diagnosis of military mTBI: review and analysis*. Retrieved from DVBIC website: http://www.dvbic.org/images/pdfs/Tech-Report-06-67-mTBI-2009.aspx.

Swerdlow, N.R., Caine, S.B. Braff, D.L., & Geyer, M.A. (1992). The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. *Journal of Psychopharmacology*, *6,* 176–190.

Takahashi, L. K., Nakashima, B.R., Hong, H., & Watanabe, K. (2005). The smell of danger: a behavioral and neural analysis of predator odor-induced fear. *Neuroscience and Biobehavioral Research, 29*(8), 1157-1167.

Tanielian, T., & Jaycox, L.H. (Eds). (2008). *Invisible wounds of war: psychological and cognitive injuries, their consequences and services to assist recovery*. Santa Monica, California. RAND Corporation MG 720-CCF.

Tanielian, T., et al. (2008). *Invisible wounds of war: summary and recommendations for addressing psychological and cognitive injuries*. Santa Monica, California: RAND Corporation, available at http://veterans.rand.org.

Taylor, S.E., Klein, L.C., Lewis, B.P., Gruenewald, T.L., Gurung, R.A.R., & Updegraff, J.A. (2000). Biobehavioral responses to stress in females: tend-and-befraind, not fight-or-flight. *Psychological Reviews, 107(3),* 411-429.

Terrio, H., Brenner, L. A., Ivins, B. J., Cho, J. M., Helmick, K., Schwab, K., et al. (2009). Traumatic brain injury screening: Preliminary findings in a US Army Brigade Combat Team. *Journal of Head Trauma Rehabilitation, 24,* 14-23.

U.S. Department of Defense and Department of Veterans Affairs, Traumatic Brain Injury Task Force. (2008). Report to the Surgeon General. Retrieved from U.S. Army Medicine website: http://www.armymedicine.army.mil/reports/tbi/TBITaskForceReportJanuary2008.pdf.

Vasterling, J.J., Proctor, S.P., Amoroso, P., Kane, R., Heeren, T., & White, R.F. (2006). Neuropsychological outcomes of Army personnel following deployment to the Iraq war. *JAMA, 296(5),* 519-529.

van Reekum, R., Cohen, T., & Wong, J. (2000). Can traumatic brain injury cause psychiatric disorders? *The Journal of Neuropsychiatry and Clinical Neurosciences, 12(3),* 316-327.

Voet, D., Voet, G. J., & Pratt, C.W. (1999). *Fundamentals of biochemistry*. John Wiley & Son, Inc, NY.

Warden, D. (2006). Military TBI during the Iraq and Afghanistan wars. *Journal of Head Trauma Rehabilitation, 21(5),* 398–402.

Watson, W.D. (2011, November). Personal Communication about mbTBI to A.M. Yarnell, Bethesda, M.D.

Weber, J.T. (2007). Experimental models of repetitive brain injuries. *Progress in Brain Research 161*, 253–61.

Weinstock, M., Matlina, E., Maor, G.I., Rosen, H., & McEwen, B.S. (1992). Prenatal stress selectivity alters the reactivity of the hypothalamic-pitutary adrenal system in the female rat. *Brain Research, 595*(2), 195-200.

Whishaw, I.Q., & Kolb, B. (Eds.). (2005). Preface. In I.Q. Whishaw and B. Kolb (Eds.), *The behavior of the laboratory rat: a handbook with tests* (pp. vii-ix). New York: Oxford.

Willner, P., Towell, A., Sampson, D., Sophocleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic mild stress (CMS) and its restoration by a tricyclic antidepressant. *Psychopharmacology*, *93***,** 358–364.

Winders, S.E., & Grunberg, N.E. (1989). Nicotine, tobacco smoke, and body weight: a review of the animal literature. *Annals of Behavioral Medicine, 11*(4), 125-133.

Wojcik, B.E., Stein, C.R., Bagg, K., Humphrey, R.J., & Orosco, J. (2010). Traumatic brain injury hospitalizations of U.S. Army soldiers deployed to Afghanistan and Iraq. *American Journal of Preventive Medicine, 38(1S),* S108-S116.

Woodcock, E.A., & Richardson, R. (2000). Effects of environmental enrichment on rate of contextual processing and discriminative ability in adult rats. *Neurobiology of Learning & Memory, 73*, 1-10.

Yarnell, A.M.**,** Chwa, A., Hamilton, K., & Grunberg, N.E. (2011, May). Warrior stress paradigm for rats. Presented at USUHS Research Week, Bethesda, Maryland.

Yerkes, R.M., & Dodson, J.D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology*, *18*, 459–482.

Zhuang, L.X., Xu, S.F., & Chun-Zhi, T.(2007). Effects of different intervention methods on behavior changes in depression rats. *Zhen Ci Yan Jiu, 32(2),* 93-97.